CHROMATOGRAM

Retention time: 6.37 (aspartic acid), 7.72 (glutamic acid), 10.81 (asparagine), 12.25 (serine), 14.20 (glutamine), 15.11 (histidine), 17.75 (glycine), 18.56 (threonine), 20.83 (arginine), 22.60 (taurine), 24.58 (alanine), 25.88 (tyrosine), 31.25 (tryptophan), 31.41 (methionine), 31.90 (valine), 32.75 (phenylalanine), 34.31 (isoleucine), 34.88 (leucine), 36.92 (lysine)
Internal standard: homoserine (16.31)
Limit of quantitation: 31 μM

KEY WORDS

plasma; derivatization

REFERENCE

Uhe,A.M.; Collier,G.R.; McLennan,E.A.; Tucker,D.J.; O'Dea,K. Quantitation of tryptophan and other plasma amino acids by automated pre-column o-phthaldialdehyde derivatization high-performance liquid chromatography: improved sample preparation, *J.Chromatogr.*, **1991**, *564*, 81–91.

SAMPLE

Matrix: blood
Sample preparation: Filter (Amicon Centrifree) while centrifuging at 1500 g for 15 min, mix 2.5 μL ultrafiltrate with 2.5 μL reagent, add 1 μL 1 mg/mL 9-fluorenylmethyl chloroformate in MeCN, mix, let stand for 2.5 min, inject the whole amount. (Prepare reagent by dissolving 3 mg o-phthalaldehyde in 50 μL MeOH, add 450 μL 0.5 M pH 10.2 sodium borate buffer, add 5 μL 3-mercaptopropionic acid. Derivatization was performed automatically and took 5 min. o-Phthalaldehyde derivatized primary amino acids and 9-fluorenylmethyl chloroformate derivatized secondary amino acids (proline and hydroxyproline).)

HPLC VARIABLES

Guard column: 20 × 2.1 5 μm Hypersil ODS
Column: two 100 × 2.1 5 μm Hypersil ODS columns in series
Mobile phase: Gradient. 15 mM pH 6.8 Sodium acetate:MeOH:10 mM pH 6.8 sodium acetate from 0:0:100 to 100:0:0 over 0.05 min, to 60:40:0 over 15 min, to 57.5:42.5:0 over 3.5 min, to 45:55:0 over 3.5 min, to 0:0:100 over 3 min, maintain at 0:0:100 for 5 min.
Column temperature: 40
Flow rate: 0.3
Injection volume: 6
Detector: F ex 230 em 450, after 20 min F ex 260 em 315

CHROMATOGRAM

Retention time: 1.8 (O-phospho-L-serine), 2 (aspartic acid), 2.5 (glutamic acid), 5 (glutathione (reduced)), 6 (asparagine), 6.2 (serine), 7.5 (glutamine), 8 (glycine), 8.5 (threonine), 8.8 (histidine), 9.2 (cystine), 9.5 (citrulline), 10.2 (taurine), 10.5 (alanine), 11.5 (arginine), 12.3 (tyrosine), 13.2 (α-amino-N-butyric acid), 15.3 (methionine), 15.5 (valine), 16 (norvaline), 16.2 (tryptophan), 16.5 (phenylalanine), 17.8 (isoleucine), 18.2 (ornithine), 18.5 (leucine), 19.5 (lysine), 20.5 (hydroxyproline), 22.3 (sarcosine), 24.5 (proline)
Limit of detection: 5 pmole

KEY WORDS

derivatization; plasma; ultrafiltrate

REFERENCE

Worthen,H.G.; Liu,H. Automatic pre-column derivatization and reversed-phase high performance liquid chromatography of primary and secondary amino acids in plasma with photo-diode array and fluorescence detection, *J.Liq.Chromatogr.*, **1992**, *15*, 3323–3341.

SAMPLE

Matrix: blood

Sample preparation: 90 μL Serum + 10 μL 2.5 mM cyclohexylalanine + 200 μL ethyl acetate + 2 μL benzoyl chloride + 6 μL triethylamine, vortex at 2500 vibrations/min for 2 min. Remove 150 μL of the ethyl acetate phase and evaporate it to dryness, dissolve the residue in 100 μL MeCN:water 70:30, inject an aliquot.

HPLC VARIABLES

Guard column: 5 μm Kromasil 100 C18
Column: 250 × 4 5 μm Kromasil 100 C18
Mobile phase: Gradient. MeCN:water from 70:30 to 95:5 over 30 min.
Flow rate: 1
Injection volume: 50
Detector: UV 274 or MS, electrospray, Finnigan MAT, TSQ 700, flow rate 1 μL/min, 2.8 kV, drying gas 140

CHROMATOGRAM

Retention time: 6.4 (lysine), 7.1 (glycine), 7.5 (alanine), 10.4 (glutamate), 8.5 (tryptophan), 8.5 (cystine), 9.4 (methionine), 11.8 (phenylalanine), 12.1 (valine), 13.3 (cysteine), 13.9 (leucine), 15.1 (isoleucine), 15.2 (naphthylalanine), 15.5 (tyrosine)
Internal standard: cyclohexylalanine (21.0)
Limit of quantitation: 10 pmol

KEY WORDS

serum; derivatization; fetal bovine serum

REFERENCE

Oehlke,J.; Brudel,M.; Blasig,I.E. Benzoylation of sugars, polyols and amino acids in biological fluids for high-performance liquid chromatographic analysis, *J.Chromatogr.B*, **1994**, *655*, 105–111.

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 500 μL 5% perchloric acid, centrifuge at 3000 rpm for 10 min. Remove the supernatant and neutralize it with 3 M potassium carbonate, centrifuge at 3000 rpm for 5 min, adjust the volume to 2 mL. (Alternatively, filter (Amicon CF-50) 4 mL plasma while centrifuging at 2500 rpm for 10 min.) 10 μL Perchloric acid extract or ultrafiltrate + 10 μL 100 mM pH 9.0 sodium bicarbonate + 40 μL freshly prepared 4 mM 4-dimethylaminoazobenzene-4'-sulfonyl chloride in MeCN, heat at 70° for 10 min, cool, make up to 500 μL with EtOH:water 70:30, centrifuge at 14000 rpm for 3 min, inject a 5 μL aliquot.

HPLC VARIABLES

Guard column: 20 × 4.6 5 μm Supelcosil LC-18 T
Column: 150 × 4.6 3 μm Supelcosil LC-18 T
Mobile phase: Gradient. A was 25 mM pH 6.8 KH_2PO_4. B was MeCN:isopropanol 80:20. A:B 80:20 for 1 min, to 77:23 over 4 min, maintain at 77:23 for 7 min, to 73:27 over 11 min, to 70:30 over 7 min, to 40:60 over 9 min, to 30:70 over 1 min, maintain at 30:70 for 5 min, return to initial conditions over 1 min, re-equilibrate for 6 min.
Flow rate: 1.5
Injection volume: 5
Detector: UV 436

CHROMATOGRAM

Retention time: 17-42

OTHER SUBSTANCES

Extracted: taurine

KEY WORDS

plasma; ultrafiltrate; derivatization

REFERENCE
Stocchi,V.; Palma,F.; Piccoli,G.; Biagarelli,B.; Cucchiarini,L.; Magnani,M. HPLC analysis of taurine in human plasma sample using the DABS-Cl reagent with sensitivity at picomole level, *J.Liq.Chromatogr.*, **1994**, *17*, 347–357.

SAMPLE
Matrix: blood
Sample preparation: Add 100 μL 200 mg/mL 5-sulfosalicylic acid in EtOH to a 1 mL tube, evaporate EtOH at 50° overnight, add 200-500 μL plasma, vortex, freeze in liquid nitrogen, store at -70°, thaw, centrifuge at 4° at 3000 g. 5 μL Supernatant + 20 μL water + 5 μL 1 mM norvaline in water + 90 μL reagent, mix thoroughly, incubate at room temperature for 3 min, add 50 μL neutralizing buffer, inject a 3 μL aliquot. (Prepare reagent stock solution by dissolving 25 mg o-phthalaldehyde in 500 μL MeOH, add 4.5 mL 100 mM pH 10.0 borate buffer, add 25 μL 3-mercaptopropionic acid. At the start of each day prepare reagent by diluting 1 part of stock solution with 20 parts 100 mM pH 10.0 borate buffer. Neutralizing buffer was 400 mM KH_2PO_4 containing 10 mL/L triethylamine.)

HPLC VARIABLES
Guard column: 10 × 2 Chrompack reverse phase
Column: 100 × 4.6 3 μm Microsphere C18 (Chrompack)
Mobile phase: Gradient. A was buffer:water:THF 50:50:0.2. B was MeOH:MeCN:buffer 35:15:50. A:B from 98:2 to 75:25 over 3.5 min, to 56:44 over 1.7 min, to 48:52 over 1.7 min, to 0:100 over 3.1 min, reset to initial conditions over 1 min.
Flow rate: 1.5
Injection volume: 3
Detector: F ex 230 em 389 (cut-off filter)

CHROMATOGRAM
Retention time: 2.3 (Asp), 4.1 (Glu), 4.8 (Asn), 5.1 (Ser), 5.8 (Gln), 6.2 (Gly), 6.4 (Thr), 6.6 (His), 6.8 (Cit), 7.0 (1-methylhistidine), 7.2 (3-methylhistidine), 7.4 (Ala), 7.6 (Tau), 7.7 (Arg), 8.5 (Tyr), 8.7 (α-aminobutyric acid), 9.8 (Val), 10.0 (Met), 10.5 (Trp), 10.7 (Phe), 11.0 (Ile), 11.2 (Orn), 11.4 (Leu), 11.6 (Lys)
Internal standard: norvaline (10.3)
Limit of quantitation: 5000 nM

KEY WORDS
plasma

REFERENCE
Teerlink,T.; Van Leeuwen,P.A.M.; Houdijk,A. Plasma amino acids determined by liquid chromatography within 17 minutes, *Clin.Chem.*, **1994**, *40*, 245–249.

SAMPLE
Matrix: blood
Sample preparation: 5 μL Plasma + 200 μL 20 mM pH 8.0 phosphate buffer, filter (Advantec Q0100, molecular mass cutoff), inject a 20 μL aliquot of the ultrafiltrate.

HPLC VARIABLES
Column: 150 × 4 5 μm Capcell C18 SG120
Mobile phase: 20 mM pH 7.5 phosphate buffer
Column temperature: 50
Flow rate: 0.4
Injection volume: 20
Detector: chemiluminescence following post-column reaction. The column effluent mixed with 5 mM niacinamide adenine dinucleotide in 20 mM pH 7.0 phosphate buffer pumped at 0.15 mL/min and 5 mM luminol in carbonate buffer pumped at 0.15 mL/min and this mixture flowed through an immobilized-enzyme reactor at 50°. The effluent from the re-

actor mixed with 20 mM potassium hexacyanoferrate(III) in water pumped at 0.4 mL/min and this mixture flowed through a 90 cm × 0.5 mm ID tube to the detector. (The carbonate buffer was 400 mM sodium carbonate containing 400 mM sodium bicarbonate, pH 10.5. Prepare the immobilized enzyme reactor as follows. Wash 1 g 13 μm poly(vinyl alcohol) beads (GS-520, Showa Denko, Tokyo) with 50 mL dry acetone, suspend in 20 mL dry acetone:pyridine 50:50 with vigorous stirring, add 1 mL 2,2,2-trifluoroethanesulfonyl chloride dropwise over 2 min, stir for 10 min, wash beads with 10 mL acetone, wash beads with 20 mL 1 mM HCl, slurry pack in a 50 × 4 column, circulate enzyme solution through the column at 0.2 mL/min for 4 h, monitor the immobilization process at 380 nm. The enzyme solution consisted of 5 mg (325 U) LeuDH (from Bacillus stearothermophilus, Unitika, Osaka) and 5 mg (280 U) NAOD (from Bacillus megaterium, Unitika, Osaka) in 10 mL 100 mM pH 7.0 phosphate buffer. When not in use store the reactor in 5 mM nicotinamide adenine dinucleotide in 20 mM pH 7.0 phosphate buffer at 5°.)

CHROMATOGRAM

Retention time: 8 (valine), 10.8 (isoleucine), 11.6 (leucine)
Limit of detection: 100 nM

KEY WORDS

post-column reaction; plasma; ultrafiltrate; immobilized enzyme reactor

REFERENCE

Kiba,N.; Oyama,Y.; Kato,A.; Furusawa,M. Postcolumn co-immobilized leucine dehydrogenase-NADH oxidase reactor for the determination of branched-chain amino acids by high-performance liquid chromatography with chemiluminescence detection, *J.Chromatogr.A*, **1996**, *724*, 354–357.

SAMPLE

Matrix: blood
Sample preparation: 500 μL Plasma + 500 μL MeCN, vortex, let stand for 10 min, centrifuge at 12000 rpm for 15 min. Remove a 10 μL aliquot of the supernatant and add it to 70 μL 200 mM pH 8.8 borate buffer, vortex, add 20 μL 3 mg/mL 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (Waters) in MeCN, vortex immediately, heat at 50° for 10 min, dilute (if necessary), inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm YMC AP303 300 Å ODS (YMC)
Mobile phase: Gradient. A was MeCN:water:buffer 2:96:2. B was MeCN:water:buffer 60:38:2. A:B 100:0 for 2 min, to 70:30 over 40 min, maintain at 70:30 for 10 min. (Prepare buffer by adjusting the pH of 1 M NaH_2PO_4 to 3.0 with phosphoric acid.)
Flow rate: 1.5
Detector: E, Bioanalytical Systems LC-4B, Model MF-1000 glassy carbon working electrode +1.1 V, stainless steel counter electrode, Ag/AgCl reference electrode

CHROMATOGRAM

Retention time: 15.2 (ammonia), 16.8 (His), 18.5 (Ser), 19.2 (Arg), 19.5 (Gly), 20.8 (Asp), 22 (Glu), 23.2 (Thr), 24.8 (Ala), 27.2 (Pro), 33 (Cys), 34.8 (Lys), 36.8 (Met), 36.8 (Tyr), 44.8 (Ile), 45.6 (Leu), 47.2 (Phe), 50.4 (Trp)
Limit of detection: 250 nM
Limit of quantitation: 500 nM

KEY WORDS

plasma; derivatization; cow; human

REFERENCE

Li,G.D.; Krull,I.S.; Cohen,S.A. Electrochemical activity of 6-aminoquinolyl urea derivatives of amino acids and peptides. Application to high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.A*, **1996**, *724*, 147–157.

SAMPLE

Matrix: blood, CSF

Sample preparation: Plasma. For each volume of plasma add 4 volumes of MeOH, centrifuge at 11600 g for 5 min. Remove a 10 μL aliquot and add it to 5 μL phthaldialdehyde/β-mercaptoethanol derivatizing reagent (Fluoraldehyde, Pierce) (use fresh reagent), allow to react at room temperature for 1 min, add 100 μL THF:100 mM sodium acetate 5:95 adjusted to pH 7.2 with glacial acetic acid, inject a 20 μL aliquot. CSF. Add an equal volume of MeOH to the CSF, centrifuge at 11600 g for 5 min. Remove a 10 μL aliquot and add it to 5 μL phthaldialdehyde, allow to react at room temperature for 1 min, add 100 μL THF:100 mM sodium acetate 5:95 adjusted to pH 7.2 with glacial acetic acid, inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: 10 × 3 Spherisorb 5 ODS

Column: 50 × 4.6 Spherisorb 5 ODS

Mobile phase: Gradient. A was THF:100 mM sodium acetate 5:95 adjusted to pH 7.2 with glacial acetic acid. B was MeOH:THF 95:5. A:B from 10:90 to 0:100 over 13 min (sic), maintain at 0:100 for 4 min, return to initial conditions over 1 min.

Column temperature: 43

Flow rate: 1.5

Injection volume: 20

Detector: F (wavelengths not specified)

CHROMATOGRAM

Retention time: 0.5 (Asp), 0.7 (Glu), 1.2 (Tau), 1.5 (Ser), 1.8 (Gln), 2.2 (His), 2.3 (Gly), 3.0 (Thr), 3.1 (Asn), 3.2 (Ala), 3.3 (Arg), 3.8 (Tyr), 5.2 (Met), 5.4 (Val), 5.8 (Trp), 6.0 (Phe), 5.5 (Ile), 5.7 (Leu), 8.3 (Orn), 8.5 (Lys)

Limit of detection: 10 nM

KEY WORDS

plasma; Thr; Gly; His co-elute; derivatization

REFERENCE

Begley,D.J.; Reichel,A.; Ermisch,A. Simple high-performance liquid chromatographic analysis of free primary amino acid concentrations in rat plasma and cisternal cerebrospinal fluid, *J.Chromatogr.B*, **1994**, *657*, 185–191.

SAMPLE

Matrix: blood, CSF, plants, tissue

Sample preparation: Seeds, serum, CSF. Homogenize seeds in 100 mL ice-cold water, centrifuge at 4° at 25000 g for 30 min. Mix seed homogenate, serum, or CSF with an equal volume of 10% trichloroacetic acid, centrifuge at 4° at 14000 g for 30 min, filter (0.2 μm) the supernatant. Dilute the supernatant with an equal volume of water. Remove a 20 (seed) or 30 (serum, CSF) μL aliquot and add it to 1 mL 25 mM pH 9.6 borate buffer, add 1 mL acetone, add 15 μL 100 μg/mL D-aminovaleric acid, add 100 μL 10 mM 9-fluorenylmethyl chloroformate in acetone (freshly prepared), vortex for 30 s, let stand at room temperature for 10 min, add 2 mL hexane:ethyl acetate 50:50, vortex for 30 s, inject a 20 μL aliquot of the aqueous layer. Tissue. Sonicate rat sensorimotor cortex with 2 mL ice-cold PBS with two 10 s bursts at 0°. Remove a 200 μL aliquot of the homogenate and add it to 100 μL 10% trichloroacetic acid, mix, centrifuge at 4° at 25000 g for 30 min. Remove a 30 μL aliquot and add it to 1 mL 25 mM pH 9.6 borate buffer, add 1 mL acetone, add 15 μL 100 μg/mL D-aminovaleric acid, add 100 μL 10 mM 9-fluorenylmethyl chloroformate in acetone (freshly prepared), vortex for 30 s, let stand at room temperature for 10 min, add 2 mL hexane:ethyl acetate 50:50, vortex for 30 s, inject a 20 μL aliquot of the aqueous layer.

HPLC VARIABLES

Column: Nova-Pak C18

Mobile phase: Gradient. MeCN:50 mM pH 3.65 sodium acetate buffer from 31:69 to 48:52 over 19 min, to 70:30 over 3 min (non-linear gradient), return to initial conditions over 8 min.
Flow rate: 1
Injection volume: 20
Detector: F ex 254 em 315

CHROMATOGRAM

Retention time: 2.5 (arginine), 3.5 (aspartate, serine), 4.5 (glutamate), 5 (threonine), 6.2 (glycine), 8 (alanine), 8.5 (tyrosine), 9.5 (gamma-aminobutyric acid), 10 (proline), 11.5 (methionine), 12.5 (valine), 14.5 (phenylalanine), 15.4 (isoleucine), 15.7 (leucine), 17.5 (β-N-methylamino-L-alanine), 18 (histidine), 18.5 (lysine, cystine)
Internal standard: D-aminovaleric acid (11)
Limit of detection: 0.6 pmole

KEY WORDS

derivatization; rat; monkey; brain; serum; seeds

REFERENCE

Kisby,G.E.; Roy,D.N.; Spencer,P.S. Determination of β-*N*-methylamino-L-alanine (BMAA) in plant (*Cycas circinalis* L.) and animal tissue by precolumn derivatization with 9-fluorenylmethyl chloroformate (FMOC) and reversed-phase high-performance liquid chromatography, *J.Neurosci.Methods*, **1988**, *26*, 45–54.

SAMPLE

Matrix: blood, CSF, tissue
Sample preparation: Blood. Mix plasma or whole blood with an equal volume of 0.5% sodium dodecyl sulfate, let stand for 15 min, add 2 volumes of 6% perchloric acid, add norleucine, centrifuge. Remove a 100 μL aliquot of the supernatant and add it to 25 μL buffer, add 125 μL freshly-prepared 2.5 μL/mL 1-naphthyl isocyanate in acetone, mix, let stand for 45 s, add 1 mL cyclohexane, vortex vigorously, discard the cyclohexane layer, repeat the wash twice more, centrifuge the aqueous layer, inject a 20 μL aliquot of the supernatant. CSF. Mix 1 volume of 60% perchloric acid with 20 volumes of CSF, centrifuge. Remove a 100 μL aliquot of the supernatant and add it to 25 μL buffer, add 125 μL freshly-prepared 2.5 μL/mL 1-naphthyl isocyanate in acetone, mix, let stand for 45 s, add 1 mL cyclohexane, vortex vigorously, discard the cyclohexane layer, repeat the wash twice more, centrifuge the aqueous layer, inject a 20 μL aliquot of the supernatant. Tissue. Homogenize brain tissue with 9 volumes 3% perchloric acid, centrifuge at 9000 g for 3 min. Remove the supernatant and neutralize it with NaOH, add 3 volumes of water, add norleucine. Remove a 100 μL aliquot and add it to 25 μL buffer, add 125 μL freshly-prepared 2.5 μL/mL 1-naphthyl isocyanate in acetone, mix, let stand for 45 s, add 1 mL cyclohexane, vortex vigorously, discard the cyclohexane layer, repeat the wash twice more, centrifuge the aqueous layer, inject a 5 μL aliquot of the supernatant. (Prepare buffer by adjusting the pH of 1 M boric acid to 6.25 with NaOH. Dry acetone over anhydrous sodium sulfate.)

HPLC VARIABLES

Column: 250 × 4.6 5 μm Absorbosphere HS C18
Mobile phase: Gradient. A was MeCN:MeOH:buffer:water 2.5:12.5:4.5:80.5. B was MeCN:buffer:water 45:4:51. C was MeCN:water 70:30. A:B:C 100:0:0 for 45 min, to 0:100:0 over 150 min, to 0:0:100 (step gradient), maintain at 0:0:100 for 10 min, re-equilibrate at initial conditions for 30 min. (Buffer was 100 mM NaH_2PO_4 containing 100 mM sodium acetate, adjusted to pH 5.4 with phosphoric acid.)
Flow rate: 1
Injection volume: 5-20
Detector: F ex 228 em 320 (cut-off filter), UV 225

CHROMATOGRAM

Retention time: 27 (Asp), 34 (Glu), 44 (Asn), 50 (Ser), 57 (Gly), 60 (Gln), 65 (Tau), 69 (His), 78 (Thr), 81 (Pro), 83 (Ala), 88 (Arg), 90 (GABA), 95 (ammonia), 105 (glutathione), 109 (Tyr), 116 (Val), 118 (Met), 130 (Ile), 131 (Leu (some interference from matrix)), 136 (Phe), 139 (Trp), 140 (Cys), 154 (Orn), 158 (Lys)

Internal standard: norleucine (133)

KEY WORDS

derivatization; plasma; whole blood; brain; human; mouse

REFERENCE

Neidle,A.; Banay-Schwartz,M.; Sacks,S.; Dunlop,D.S. Amino acid analysis using 1-naphthylisocyanate as a precolumn high performance liquid chromatography derivatization reagent, *Anal.Biochem.*, **1989**, *180*, 291–297.

SAMPLE

Matrix: blood, food, peptides, plants, tissue

Sample preparation: Hydrolyze peptide with 6 M HCl containing 0.2% 3,3'-thiodipropionic acid at 110° for 24 h, evaporate to dryness, reconstitute with 50-200 μL 0.1% HCl containing 0.2% 3,3'-thiodipropionic acid. Homogenize (Ultra-Turrax) 0.1-1 g food, tissue, plant material, lyophilized plasma, or lyophilized tissue in 10 mL 250 nM IS in 100 mM HCl containing 0.2% 3,3'-thiodipropionic acid at 20000 rpm for 2 min, sonicate for ≤30 min, centrifuge at 5000 g for 20 min, discard fat layer, filter (Millipore ultrafiltration insert (MW cutoff 5000) prewashed with 200 μL 100 mM HCl containing 0.2% 3,3'-thiodipropionic acid) 3 mL supernatant while centrifuging at 3500 g for 1 h. Mix 20 μL deproteinized sample (or 10 μL peptide hydrolysate) with 180 μL buffer, vortex, add 200 μL reagent, mix, heat at 70° for 15 min with mixing at 1 min and 12 min, cool in an ice bath for 5 min, centrifuge at 10000 g for 10 s, add 400 μL diluent, mix thoroughly, centrifuge at 15000 g for 5 min, inject a 10 μL aliquot of the supernatant. (Prepare buffer by dissolving 630 mg sodium bicarbonate in 40 mL water, adjusting pH to 8.6 with NaOH, and making up to 50 mL with water. Prepare reagent by sonicating 40 mg dabsyl chloride in 10 mL acetone for 10 min, then filtering into brown vials and storing at -20°. Prepare diluent by mixing 50 mL MeCN, 25 mL EtOH, and 25 mL mobile phase A.)

HPLC VARIABLES

Guard column: present but not specified

Column: 150 × 3.9 4 μm Novapak C18

Mobile phase: Gradient. A was DMF:9 mM NaH_2PO_4 containing 0.16% triethylamine, adjusted to pH 6.55 with phosphoric acid. B was MeCN:water 80:20. A:B 92:8 for 2 min, to 80:20 over 5 min (Waters convex curve 5), to 65:35 over 28 min (Waters concave curve 7), to 50:50 over 10 min, to 0:100 over 21 min, maintain at 0:100 for 11 min, return to initial conditions over 0.5 min, re-equilibrate for 12.5 min.

Column temperature: 50

Flow rate: 1

Injection volume: 10

Detector: UV 436

CHROMATOGRAM

Retention time: 13.95 (O-phosphoserine), 14.94 (aspartic acid), 15.15 (O-phosphothreonine), 15.91 (glutamic acid), 16.39 (carboxymethylcysteine), 16.89 (S-sulfocysteine), 17.10 (β-aminoadipic acid), 22.51 (hydroxyproline), 23.13 (asparagine), 24.72 (glutamine), 25.35 (citrulline), 26.17 (serine), 27.43 (phosphoethanolamine), 27.69, 28.15 (methionine sulfoxide (diastereomers)), 28.45 (threonine), 28.94 (glycine), 28.94 (1- and 3-methylhistidine), 29.38 (arginine), 30.65 (alanine), 30.92 (β-alanine), 31.52 (anserine), 32.31 (taurine), 32.58 (sarcosine), 32.75 (α-aminobutyric acid), 33.27 (gamma-aminobutyric acid), 33.99 (proline), 34.40 (β-aminoisobutyric acid), 35.55 (valine), 37.60 (methionine), 39.35 (isoleucine), 40.03 (leucine), 40.51 (tryptophan), 41.57 (phenylalanine), 42.05 (ammonia), 43.62 (lanthionine), 44.00 (agmatine), 44.76 (2-aminoethanol), 44.76 (cystathionine), 45.65 (cyste-

ine), 46.83 (homocysteine), 48.02 (1-amino-2-propanol), 50.04 (hydroxylysine), 51.02 (ornithine), 51.58 (lysine), 52.10 (histidine), 52.38 (carnosine), 52.59 (ethylamine), 54.30 (tyrosine), 57.22 (pyrrolidine), 57.22 (tryptamine), 57.79 (isobutylamine), 58.34 (3,4-dihydroxyphenylalanine), 59.20 (phenylethylamine), 59.94 (methylbutylamine), 62.16 (putrescine), 63.29 (cadaverine), 63.87 (histamine), 63.87 (cystamine), 65.00 (serotonin), 67.31 (tyramine), 67.98 (spermidine), 68.49 (norepinephrine), 69.05 (dopamine), 70.61 (epinephrine), 71.94 (spermine)
Internal standard: norleucine (40.90), norvaline (35.06)
Limit of detection: 0.12-0.52 pmole
Limit of quantitation: 0.4-1.5 pmole

KEY WORDS
rinse glass and plasticware with 70% EtOH and water and dry before use; derivatization; cheese; meat; sausage; fish; plasma

REFERENCE
Krause,I.; Bockhardt,A.; Neckermann,H.; Henle,T.; Klostermeyer,H. Simultaneous determination of amino acids and biogenic amines by reversed-phase high-performance liquid chromatography of the dabsyl derivatives, *J.Chromatogr.A*, **1995**, *715*, 67–79.

SAMPLE
Matrix: blood, juice, urine
Sample preparation: Serum, urine. 400 μL Serum or urine + 50 μL 30% 5-sulfosalicylic acid + 50 μL 0.52 mM L-homo-Arg, centrifuge at 6000 g. Remove a 2 μL aliquot and add it to 5 μL 0.4 N pH 10.4 sodium borate buffer and 1 μL reagent, mix for 2 min, inject a 7 μL aliquot. Juice. Centrifuge filtered (paper) apple juice at 1650 g, remove a 1 mL aliquot and add it to 31.3 μL 1.6 mM L-homo-Arg in 100 mM HCl, adjust pH to 2.0 with 2 M HCl, add to a 50 × 10 column of Dowex 50W-X8 cation-exchanger, wash with water, elute with 30 mL 4 M aqueous ammonia, evaporate eluate to dryness, dissolve the residue in 2 mL 100 mM HCl. Remove a 2 μL aliquot and add it to 5 μL 0.4 N pH 10.4 sodium borate buffer and 1 μL reagent, mix for 2 min, inject a 7 μL aliquot. (Reagent was 260 mM N-isobutyryl--L-cysteine and 170 mM o-phthaldialdehyde in 1 M pH 10.4 potassium borate buffer (fluoraldehyde, Pierce) (Chromatographia 1991, 32, 383).)

HPLC VARIABLES
Guard column: 20 × 2.1 5 μm Hypersil ODS
Column: 250 × 4 5 μm Hypersil ODS
Mobile phase: Gradient. A was 3.13 g sodium acetate trihydrate in 990 mL water adjusted to pH 5.95 with 10% acetic acid, make up to 1 L. B was MeCN:MeOH 50:600. A:B from 100:0 to 46.5:53.5 over 75 min, re-equilibrate at 100:0 for 10 min.
Column temperature: 25
Flow rate: 1
Injection volume: 7
Detector: F ex 230 em 445 (280 nm cut-off filter)

CHROMATOGRAM
Retention time: 18.89 (L-Asp), 20.09 (D-Asp), 25.87 (L-Glu), 27.54 (D-Glu), 26.95 (L-Asn), 29.30 (D-Asn), 28.46 (L-Ser), 30.61 (D-Ser), 32.41 (L-Gln), 34.01 (D-Gln), 34.59 (L-Thr), 36.78 (D-Thr), 36.21 (Gly), 37.76 (L-His), 39.15 (D-His), 41.95 (L-Ala), 45.27 (D-Ala), 43.29 (L-Arg), 44.66 (D-Arg), 48.29 (L-Tyr), 50.87 (D-Tyr), 55.36 (L-Val), 60.71 (D-Val), 56.30 (L-Met), 59.65 (D-Met), 58.53 (L-Trp), 63.08 (D-Trp), 61.80 (L-Phe), 64.39 (D-Phe), 62.33 (L-Ile), 67.56 (D-Ile), 66.45 (L-Leu), 69.87 (D-Leu), 71.41 (L-Lys), 72.69 (D-Lys)
Internal standard: L-hono-Arg (47.5)

KEY WORDS
serum; human; dog; apple; chiral; derivatization

REFERENCE

Brückner,H.; Haasmann,S.; Langer,M.; Westhauser,T.; Wittner,R.; Godel,H. Liquid chromatographic determination of D- and L-amino acids by derivatization with o-phthaldialdehyde and chiral thiols Applications with reference to biosciences, *J.Chromatogr.A*, **1994**, *666*, 259–273.

SAMPLE

Matrix: blood, protein

Sample preparation: Plasma. Mix plasma vigorously with 3 volumes MeCN, centrifuge at 12000 g for 3 min, evaporate an aliquot of the supernatant to dryness under reduced pressure, reconstitute with buffer. Remove a 5 μL aliquot and add it to 5 μL 4.16 mg/mL 9-fluorenylmethyl chloroformate in MeCN, mix, let stand for 1.5 min, add 5 μL reagent, mix, let stand for 3.5 min, add 5 μL MeCN:water:acetic acid 80:12:8, mix, inject an aliquot. Protein. Hydrolyse protein with 500 μL 6 M HCl at 110° for 24 h, evaporate to dryness under reduced pressure, add 10 μL triethylamine:EtOH:water 40:40:20, evaporate to dryness, reconstitute with 5 μL buffer, add 5 μL 4.16 mg/mL 9-fluorenylmethyl chloroformate in MeCN, mix, let stand for 1.5 min, add 5 μL reagent, mix, let stand for 3.5 min, add 5 μL MeCN:water:acetic acid 80:12:8, mix, inject an aliquot. (Buffer was 200 mM boric acid adjusted to pH 8.5 with 5 M NaOH. Prepare reagent by mixing 170 μL 850 mM NaOH, 75 μL 500 mM hydroxylamine hydrochloride, and 5 μL 2-(methylthio)ethanol.)

HPLC VARIABLES

Guard column: 15 × 3.2 7 μm Newguard ODS

Column: 150 × 4.6 3 μm Spherisorb ODS-2

Mobile phase: Gradient. A was MeOH:20 mM pH 6.5 $(NH_4)H_2PO_4$ 15:85. B was MeCN: water 90:10. A:B 82:18 for 2 min, to 77:23 over 1 min, maintain at 77:23 for 7 min, to 64: 36 over 10 min, to 52:48 over 1 min, maintain at 52:48 for 5 min, to 45:55 over 2 min, to 1:99 over 1 min (plasma). A:B 82:18 for 2 min, to 77:23 over 1 min, maintain at 77:23 for 3 min, to 60:40 over 10 min, to 55:45 over 1 min, maintain at 55:45 for 3 min, to 45:55 over 2 min, to 1:99 over 1 min (protein hydrolysate).

Column temperature: 35

Flow rate: 1

Detector: F ex 263 em 313, UV 263

CHROMATOGRAM

Retention time: 3 (phosphoserine), 3.5 (aspartic acid), 3.7 (glutamic acid), 4 (α-aminoadipic acid), 4.2 (S-carboxymethylcysteine), 6.7 (hydroxyproline), 7.5 (asparagine), 8.5 (glutamine), 8.7 (citrulline), 9 (serine), 9.5 (histidine), 10 (glycine), 10.6 (threonine), 11.2 (β-alanine), 11.7 (alanine), 12.3 (taurine), 13 (proline), 14.5 (tyrosine), 15 (α-aminobutyric acid), 15.8 (arginine), 17 (homoarginine), 17.7 (valine), 18.3 (methionine), 20.5 (isoleucine), 21 (leucine), 21.5 (norleucine), 22 (phenylalanine), 23 (cystathionine), 26.3 (ornithine), 27 (lysine, using plasma gradient.)

Limit of detection: 50 fmole

KEY WORDS

derivatization; plasma

REFERENCE

Haynes,P.A.; Sheumack,D.; Greig,L.G.; Kibby,J.; Redmond,J.W. Applications of automated amino acid analysis using 9-fluorenylmethyl chloroformate, *J.Chromatogr.*, **1991**, *588*, 107–114.

SAMPLE

Matrix: blood, tissue

Sample preparation: Plasma. Mix 9 volumes of plasma with 1 volume of 35% 5-sulfosalicylic acid, centrifuge at 2000 g for 10 min. Neutralize the supernatant with 10 M KOH, dilute with 2 volumes of water. Mix an aliquot with an equal volume of reagent, inject a 20 μL aliquot within 1 min. Tissue. Homogenize tissue with four volumes 5% 5-sulfo-

salicylic acid, centrifuge at 5000 g for 10 min, neutralize the supernatant with 10 M KOH. Mix an aliquot with an equal volume of reagent, inject a 20 μL aliquot within 1 min. (Prepare reagent each day by dissolving 35 mg o-phthalaldehyde in 500 μL 95% EtOH and adding this mixture to 50 mL 100 mM pH 10.4 borate buffer, add 100 μL 2-mercaptoethanol.)

HPLC VARIABLES

Guard column: 37-50 μm Bondapak C18/Corasil
Column: 150 × 3.9 4 μm Nova-Pak C18
Mobile phase: Gradient. A was THF:water 3:97 containing 100 mM potassium phosphate, pH 7.0. B was THF:MeCN:water 3:40:57 containing 100 mM potassium phosphate, pH 7.0. A:B 97:3 for 1.5 min, to 68:32 over 17 min (Waters curve profile 3), to 0:100 over 2 min, maintain at 0:100 for 4.5 min, return to initial conditions over 2 min, re-equilibrate for 8 min.
Column temperature: 41
Flow rate: 1
Injection volume: 20
Detector: F ex 360 em 455

CHROMATOGRAM

Retention time: 3.5 (aspartate, cysteate), 5 (cysteinesulfinate), 6 (glutamate), 10.5 (serine), 11 (glutamine), 12.5 (arginine), 13.5 (glycine), 15 (threonine, glycerophosphorylethanolamine), 15.3 (o-phosphorylethanolamine), 18.5 (alanine), 20.5 (hypotaurine), 21.5 (taurine), 22 (β- and gamma-aminobutyrate), 25 (tyrosine), 25.5 (α-aminobutyrate), 26 (methionine), 29 (ethanolamine)

KEY WORDS

plasma; human; rat; liver; kidney; heart; brain; derivatization

REFERENCE

Hirschberger,L.L.; De La Rosa,J.; Stipanuk,M.H. Determination of cysteinesulfinate, hypotaurine and taurine in physiological samples by reversed-phase high-performance liquid chromatography, *J.Chromatogr.*, **1985**, *343*, 303–313.

SAMPLE

Matrix: blood, tissue
Sample preparation: 250 μL Plasma + 5 μL 2 mM norleucine + 2 mL 10% trichloroacetic acid, mix, centrifuge at 10000 g for 10 min. Adjust the pH of the supernatant to 9.0 with KOH. Remove a 40 μL aliquot and add it to 40 μL 100 mM pH 8.3 sodium bicarbonate, add 80 μL 4 mM dabsyl chloride in MeCN, heat at 70° for 12 min (mix after 1 and 4 min), cool to room temperature for 5 min, add 440 μL EtOH:50 mM pH 7.0 sodium phosphate buffer 50:50, inject a 50 μL aliquot. Liver. Homogenize (Polytron) 200 mg liver with 5 volumes of 10% trichloroacetic acid, mix, centrifuge at 10000 g for 10 min. Adjust the pH of the supernatant to 9.0 with KOH. Remove a 40 μL aliquot and add it to 40 μL 100 mM pH 8.3 sodium bicarbonate, add 80 μL 4 mM dabsyl chloride in MeCN, heat at 70° for 12 min (mix after 1 and 4 min), cool to room temperature for 5 min, add 440 μL EtOH: 50 mM pH 7.0 sodium phosphate buffer 50:50, inject a 50 μL aliquot.

HPLC VARIABLES

Guard column: 5 μm Adsorbosphere C18
Column: 250 × 4.6 5 μm Econosphere
Mobile phase: Gradient. A was DMF:10 mM pH 6.5 citrate buffer 4:96. B was MeCN:10 mM pH 6.5 citrate buffer:DMF 67.2:28.8:4. A:B 83:17 for 5 min, to 74:26 over 18.6 min, to 63:37 over 16 min, to 19:81 over 34.7 min, to 0:100 over 0.3 min, maintain at 0:100 for 1.4 min, return to initial conditions, re-equilibrate for 14 min. (Condition column with DMF:100 mM pH 3.5 sodium citrate 20:80 at 1 mL/min for 1 h before use.)
Flow rate: 1.4
Injection volume: 50

Detector: UV 436

CHROMATOGRAM

Retention time: 28.7 (aspartate), 30.9 (cysteine), 32.7 (glutamate), 43.4 (glutamine), 43.8 (serine), 44.6 (threonine), 45.3 (arginine), 46.0 (glycine), 46.5 (alanine), 48.6 (proline), 49.5 (valine), 51.0 (methionine), 51.5 (leucine), 52.2 (isoleucine), 54 (phenylalanine), 66.4 (lysine), 67.6 (histidine), 71.5 (tyrosine)
Internal standard: norleucine (53)

KEY WORDS

derivatization; rat; plasma; liver

REFERENCE

Drnevich,D.; Vary,T.C. Analysis of physiological amino acids using dabsyl derivatization and reversed-phase liquid chromatography, *J.Chromatogr.*, **1993**, *613*, 137–144.

SAMPLE

Matrix: blood, tissue
Sample preparation: Serum. 10 μL Serum + 90 μL MeOH, mix vigorously, centrifuge at 1000 g for 5 min. Remove a 10 μL aliquot of the supernatant and add it to 10 μL 200 mM pH 8.0 borate buffer containing 4 mM tetrasodium EDTA, add 30 μL 50 mM 4-fluoro-7-nitrobenzofurazan in MeCN, heat at 60° for 5 min, cool, add 250 μL MeOH:acetic acid 99:1, filter (0.5 μm), inject a 10 μL aliquot of the filtrate. Rat brain. Homogenize (glass-Potter) rat brain tissue in 10 volume MeOH at 4°, centrifuge at 1000 g for 10 min. Remove a 10 μL aliquot of the supernatant and add it to 10 μL 200 mM pH 8.0 borate buffer containing 4 mM disodium EDTA, add 30 μL 50 mM 4-fluoro-7-nitrobenzofurazan in MeCN, heat at 60° for 5 min, cool, add 250 μL MeOH:acetic acid 99:1, filter (0.5 μm), inject a 10 μL aliquot of the filtrate. Cow brain. Homogenize (glass-Potter) rat brain tissue in 10 volume buffer at 4°, filter through gauze. Vigorously mix 10 μL filtrate with 90 μL MeOH, centrifuge at 1000 g for 5 min. Remove a 10 μL aliquot of the supernatant and add it to 10 μL 200 mM pH 8.0 borate buffer containing 4 mM disodium EDTA, add 30 μL 50 mM 4-fluoro-7-nitrobenzofurazan in MeCN, heat at 60° for 5 min, cool, add 250 μL MeOH:acetic acid 99:1, filter (0.5 μm), inject a 10 μL aliquot of the filtrate. (Buffer was 10 mM pH 7.4 N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES) containing 122 mM NaCl, 3 mM KCl, 25 mM sodium bicarbonate, 1.4 mM calcium chloride, 1.2 mM magnesium sulfate, and 0.4 mM glucose.)

HPLC VARIABLES

Guard column: Resolve C18 guard column (Waters)
Column: 250 × 4.6 Sumichiral OA-3100(S) (Sumika)
Mobile phase: MeOH containing 5 mM citric acid
Flow rate: 1 (standards) or 0.5 (biological samples)
Injection volume: 10
Detector: F ex 470 em 530

CHROMATOGRAM

Retention time: k' 1.01 (first (D) enantiomer, α = 1.52 (Leu)), k' 1.18 (first (D) enantiomer, α = 1.25 (Ile)), k' 1.29 (first (D) enantiomer, α = 1.22 (Val)), k' 2.05 (first (D) enantiomer, α = 1.31 (Ala)), k' 5.48 (first (D) enantiomer, α = 1.06 (Pro)), k' 2.13 (first (D) enantiomer, α = 1.35 (Thr)), k' 3.10 (first (D) enantiomer, α = 1.33 (Ser)), k' 2.16 (first (D) enantiomer, α = 1.36 (Phe)), k' 2.08 (first (D) enantiomer, α = 1.40 (Met)), k' 3.00 (first (D) enantiomer, α = 1.27 (Gln)), k' 4.96 (first (D) enantiomer, α = 1.57 (Lys))

KEY WORDS

derivatization; serum; human; rat; cow; brain; chiral; detailed discussion of use of other chiral columns; detailed discussion of use of other derivatizing reagents

REFERENCE

Fukushima,T.; Kato,M.; Santa,T.; Imai,K. Enantiomeric separation and sensitive determination of D,L-amino acids derivatized with fluorogenic benzofurazan reagents on Pirkle type stationary phases, *Biomed.Chromatogr.*, **1995**, *9*, 10–17.

SAMPLE

Matrix: blood, tissue, urine, ascitic fluid

Sample preparation: Homogenize liver with 5 volumes ice-cold 3% sulfosalicylic acid, centrifuge at 2000 g for 15 min. Add an equal volume of cold 6% sulfosalicylic acid to plasma, urine, or ascitic fluid, mix, centrifuge at 4° at 1800 g for 12 min. Dilute the supernatant or liver homogenate with an equal volume of 400 μM methionine sulfone in 100 mM HCl, evaporate an aliquot to dryness under reduced pressure, add 20 μL EtOH:water:triethylamine 40:40:20, evaporate to dryness under reduced pressure, reconstitute with 20 μL EtOH:triethylamine:water:phenyl isothiocyanate 70:10:10:10 (freshly prepared), let stand at room temperature for 20 min, evaporate to dryness under reduced pressure (70 mTorr) for 1.5-2 h, reconstitute with 250 μL pH 7.4 phosphate buffer, inject an aliquot.

HPLC VARIABLES

Column: 150 × 3.9 Pico-Tag C18 (Waters)

Mobile phase: Gradient. A was MeCN:buffer 6:94. B was MeCN:water 60:40. A:B from 98:2 to 54:46 over 10 min (Waters convex curve No. 5), to 0:100 over 0.5 min, maintain at 0:100 for 5 min, re-equilibrate at initial conditions for 7 min. (Buffer was 140 mM sodium acetate in water containing 500 μL/L triethylamine, pH adjusted to 6.40 with glacial acetic acid.)

Column temperature: 38

Flow rate: 1

Detector: UV 254

CHROMATOGRAM

Retention time: 1.7 (Asp), 1.9 (Glu), 2.7 (Hpro), 3.45 (Asn), 3.65 (Ser), 3.8 (Gln), 4.0 (Gly), 4.6 (His), 5.25 (Tau), 5.5 (Arg), 5.7 (Thr), 5.9 (Ala), 6.3 (Pro), 7.2 (AAB), 7.8 (Tyr), 8.4 (Val), 8.8 (Met), 9.5 (Cys), 9.75 (Ile), 9.9 (Leu), 10.9 (Phe), 11.3 (Trp), 12 (Lys)

Internal standard: methionine sulfone (6.5)

Limit of detection: 3 μM

KEY WORDS

derivatization; plasma; liver

REFERENCE

Fierabracci,V.; Masiello,P.; Novelli,M.; Bergamini,E. Application of amino acid analysis by high-performance liquid chromatography with phenyl isothiocyanate derivatization to the rapid determination of free amino acids in biological samples, *J.Chromatogr.*, **1991**, *570*, 285–291.

SAMPLE

Matrix: blood, urine

Sample preparation: Serum. 100 μL Serum + 400 μL EtOH, mix, centrifuge. Remove the supernatant and evaporate it to dryness under reduced pressure, reconstitute with 200 μL MeOH:triethylamine 95:5 (prepare fresh each day), add 10 μL phenylisothiocyanate:MeOH 12.5:87.5 (prepare fresh each day), mix, let stand at room temperature for 5 min, evaporate to dryness under reduced pressure, reconstitute with 500 μL buffer, add 200 μL dichloromethane, vortex for 1 min, centrifuge at 1200 g for 1-3 min, inject an aliquot of the aqueous layer. Urine. Add 10 μL IS solution to 50/x μL urine (x = concentration of creatinine (mM)), add 200 μL EtOH, mix, centrifuge. Remove the supernatant and evaporate it to dryness under reduced pressure, reconstitute with 200 μL MeOH:triethylamine 95:5 (prepare fresh each day), add 10 μL phenylisothiocyanate:MeOH 12.5:87.5 (prepare fresh each day), mix, let stand at room temperature for 5 min, evaporate to dryness under reduced pressure, reconstitute with 500 μL buffer, add 200 μL dichloromethane, vortex

for 1 min, centrifuge at 1200 g for 1-3 min, inject an aliquot of the aqueous layer. (Prepare buffer by dissolving 1.36 g sodium acetate trihydrate in water, adjust pH to 6.40 ± 0.01 with 2% orthophosphoric acid, make up to 1 L with water.)

HPLC VARIABLES

Column: 250 × 4.6 5 μm Hypersil-ODS

Mobile phase: Gradient. A was 1.36 g/L sodium acetate trihydrate in water, pH adjusted to 6.40 ± 0.01 with 2% orthophosphoric acid. B was 1.36 g/L sodium acetate trihydrate in MeCN:water 60:40, pH adjusted to 6.40 ± 0.01 with 2% orthophosphoric acid. A:B from 100:0 to 87:13 over 20 min, to 45:55 over 45 min, to 0:100 over 2.5 min, maintain at 0:100 for 2.5 min, return to initial conditions over 5 min, re-equilibrate for 5 min.

Flow rate: 1 for 65 min then 2

Injection volume: 20

Detector: E, EDT Research LCA 15, glassy carbon electrode +1.10 V, Ag/AgCl reference electrode

CHROMATOGRAM

Retention time: 3.82 (phosphoserine), 5.44 (aspartic acid), 6.83 (glutamic acid), 10.35 (gamma-aminoadipic acid), 12.26 (hydroxyproline), 12.45 (phosphoethanolamine), 14.52 (serine), 15.52 (glycine), 15.54 (asparagine), 16.91 (sarcosine), 17.42 (β-alanine), 18.98 (taurine), 20.48 (gamma-aminobutyric acid), 20.63 (citrulline), 21.11 (threonine), 21.75 (alanine), 22.27 (β-aminoisobutyric acid), 23.48 (proline), 23.91 (histidine), 25.71 (carnosine), 28.22 (arginine), 28.39 (1-methylhistidine, 3-methylhistidine), 28.60 (α-aminobutyric acid), 29.06 (anserine), 35.02 (tyrosine), 35.76 (valine), 37.65 (ethanolamine), 38.10 (methionine), 38.19 (cystathionine), 40.88 (cystine), 42.80 (isoleucine), 43.47 (leucine), 47.78 (hydroxylysine), 48.20 (phenylalanine), 48.46 (hydroxylysine), 49.67 (ornithine), 50.16 (tryptophan), 52.79 (lysine)

Internal standard: norleucine (45.07)

Limit of quantitation: 1 μM

KEY WORDS

derivatization; serum

REFERENCE

Sherwood,R.A.; Titheradge,A.C.; Richards,D.A. Measurement of plasma and urine amino acids by high-performance liquid chromatography with electrochemical detection using phenylisothiocyanate derivatization, *J.Chromatogr.*, **1990**, *528*, 293–303.

SAMPLE

Matrix: blood, urine

Sample preparation: Plasma. 400 μL Plasma + 100 μL 100 mM dithiothreitol, heat at 37° for 15 min, add 100 μL 150 mg/mL sulfosalicylic acid, mix, let stand at room temperature for 30 min, centrifuge at 4000 g for 15 min, inject a 30 μL aliquot. Urine. 400 μL Urine + 30 μL 100 mM dithiothreitol + 70 μL 300 mM pH 8.5 Tris buffer, mix, heat at 37° for 15 min, add 100 μL 100 mg/mL sulfosalicylic acid, mix, let stand at room temperature for 30 min, centrifuge at 4000 g for 15 min, inject a 30 μL aliquot.

HPLC VARIABLES

Guard column: 10 × 3.2 7 μm G18-013 C18 (Brownlee)

Column: 100 × 3.2 3.2 Velosep RP-18 (Brownlee, Applied Biosystems)

Mobile phase: MeOH:buffer 4.3:100 (Buffer was 16 mM NaH_2PO_4, 19 mM phosphoric acid, and 8 mM octyl sulfate, pH 2.36 ± 0.02. Use a 30 × 3.2 5 μm Brownlee SS-GU silica column before the injector.)

Flow rate: 0.8

Injection volume: 30

Detector: UV 324 following post-column reaction. The effluent from the column mixed with the reagent pumped at 0.4 mL/min and the mixture flowed through a 2 m × 0.5 mm stainless steel coil to the detector. (Prepare reagent by mixing (at 4°) 3 mL 10 mM 4,4'-

dithiopyridine in MeOH:10 mM HCl 3:97 with 300 mL 300 mM Tris base containing 1 mM EDTA (adjusted to pH 8.5 with phosphoric acid). Sparge with helium before use, keep in an ice bath during use.)

CHROMATOGRAM

Retention time: 5.4 (cysteine), 7.2 (glutathione), 10.4 (gamma-glutamylcysteine), 13.2 (cysteinylglycine), 15.9 (homocysteine)

Limit of detection: 50 nM

KEY WORDS

derivatization; plasma; post-column reaction

REFERENCE

Andersson,A.; Isaksson,A.; Brattström,L.; Hultberg,B. Homocysteine and other thiols determined in plasma by HPLC and thiol-specific postcolumn derivatization, *Clin.Chem.*, **1993**, *39*, 1590–1597.

SAMPLE

Matrix: bulk

Sample preparation: Dissolve 0.5 mmole amino acid and 0.5 mmole (1S)-(+)-10-camphorsulfonyl chloride in 10 mL ether, add 10 mL 1 M NaOH, stir vigorously for 1 h, acidify with 1 M HCl, extract with ether. Evaporate the extract to dryness and reconstitute with chloroform containing 0.5 mmole p-nitrobenzyl bromide, reflux for 30 min, wash with water, dry over anhydrous sodium sulfate, evaporate to dryness.

HPLC VARIABLES

Column: 250 × 2 MicroPak Si-5

Mobile phase: Isooctane:isopropanol 98.5:1.5

Flow rate: 0.5

Detector: UV 254

CHROMATOGRAM

Retention time: 7.0 (L-isoleucine), 7.6 (D-isoleucine, L-leucine), 8.6 (D-leucine), 11.0 (L-phenylalanine), 12.4 (D-phenylalanine), 17.0 (L-alanine), 22.3 (D-alanine)

KEY WORDS

derivatization; normal phase; chiral

REFERENCE

Furukawa,H.; Sakakibara,E.; Kamei,A.; Ito,K. Separation of L- and D-amino acids as diastereomeric derivatives by high performance liquid chromatography, *Chem.Pharm.Bull.*, **1975**, *23*, 1625–1626.

SAMPLE

Matrix: bulk

Sample preparation: Dissolve 1 mmole amino acid in 10 mL diethyl ether, add 20 mL 1 M NaOH, stir vigorously at 0°, add 2 mmole (1S)-(+)-10-camphorsulfonyl chloride in 30 mL ether dropwise, stir at room temperature for 1 h. Remove the aqueous layer and wash it twice with diethyl ether, acidify the aqueous layer with concentrated HCl, extract with diethyl ether. Dry the organic extract over anhydrous sodium sulfate, evaporate to dryness, reconstitute with 10 mL DMF, add 1 drop trimethylamine, add 1.1 mmole p-nitrobenzyl bromide, heat at 55° for 2 h, dilute with 40 mL chloroform, wash with water, dry over anhydrous sodium sulfate, evaporate to dryness, reconstitute with chloroform, inject an aliquot.

HPLC VARIABLES

Column: 250 × 2.2 10 μm MicroPak-NH2

Mobile phase: Isooctane:dichloromethane:isopropanol 79:16:5

Flow rate: 0.4

Detector: UV 254

CHROMATOGRAM

Retention time: 3.9 (L-leucine), 4.4 (L-isoleucine, D-leucine), 5.0 (D-isoleucine), 6.2 (L-phenylalanine), 7.2 (L-alanine), 7.4 (L-methionine), 9.3 (D-alanine), 8.5 (D-phenylalanine), 10.0 (D-methionine), 12.8 (L-glutamic acid), 16.8 (D-glutamic acid), 29.2 (L-tryptophan), 33.2 (L-tyrosine), 47.2 (D-tyrosine), 49.6 (D-tryptophan)

KEY WORDS

derivatization; normal phase; chiral

REFERENCE

Furukawa,H.; Mori,Y.; Takeuchi,Y.; Ito,K. Separation of L- and D-amino acids as diastereomeric derivatives by high-performance liquid chromatography, *J.Chromatogr.*, **1977**, *136*, 428–431.

SAMPLE

Matrix: bulk

Sample preparation: Dissolve 1 mg amino acids in 100 μL 100 mM pH 9.0 sodium bicarbonate buffer, add 100 μL 2 mM dabsyl chloride in acetone, if necessary adjust pH to 9.0 with 100 mM NaOH, heat at 70° for 10 min, evaporate to dryness under reduced pressure, reconstitute with EtOH:water 70:30, inject a 5-25 μL aliquot. (Purify dabsyl chloride by dissolving 1 g in 100 mL boiling acetone, filtering (sintered glass), cooling to -20° overnight, and collecting the needle-shaped crystals.)

HPLC VARIABLES

Column: Zorbax ODS
Mobile phase: Gradient. MeCN:buffer from 20:80 to 70:30 over 25 min, maintain at 70:30.
Flow rate: 1.2
Injection volume: 5-25
Detector: UV 436

CHROMATOGRAM

Retention time: 10 (cysteic acid), 12 (carboxymethylcysteine), 13 (Asp, Ser), 14 (Glu), 14.5 (Thr), 15 (Gly), 16 (Arg), 17 (Ala), 19 (Met), 20 (Pro), 20.5 (Val), 21.5 (Phe), 22 (ammonia), 23 (Leu, Ile), 26.5 (His), 27.5 (Lys), 30 (Tyr)
Limit of detection: 2-5 pmole

KEY WORDS

derivatization

REFERENCE

Chang,J.-Y.; Knecht,R.; Braun,D.G. Amino acid analysis at the picomole level. Application to the C-terminal sequence analysis of polypeptides, *Biochem.J.*, **1981**, *199*, 547–555.

SAMPLE

Matrix: bulk

Sample preparation: Prepare a 5 mg/mL solution in 1 M HCl or MeOH:water 25:75 depending on solubility, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 300 mm long μBondapak C18
Mobile phase: 8 mM (S)-Proline containing 4 mM cupric acetate, adjusted to pH 5 with NaOH
Flow rate: 3
Injection volume: 20
Detector: F ex 365 em 455 following post-column reaction. The column effluent mixed with the reagent pumped at 1.5 mL/min and this mixture flowed to the detector. (Prepare the

reagent by adding 7.5 mL 1% o-phthalaldehyde in EtOH:water 95:5, 1.8 mL Brij-35 surfactant, 200 μL mercaptoethanol, and 570 mg EDTA to 285 mL 50 mM pH 9.5 boric acid buffer, adjust pH to 10-11 with NaOH.)

CHROMATOGRAM
Retention time: 4.54 (R-valine), 10.03 (S-valine), 11.00 (R-tyrosine), 20.24 (S-tyrosine)
Limit of detection: 0.01% (of major enantiomer): chiral

KEY WORDS
derivatization; post-column reaction

REFERENCE
Cotter,M.L.; Naldi,R.; Shaw,C.; Park,S.; Heavner,G.A. Detection and quantitation of low levels of protected and unprotected (*R*)-amino acids in the synthesis of thymopentin, an immunoregulatory peptide, *J.Pharm.Sci.*, **1985**, *74*, 489–491.

SAMPLE
Matrix: bulk
Sample preparation: Dissolve 500 μg amino acids in 1 mL pH 10.65 triethylamine/acetic acid buffer, remove a 100 μL aliquot and add it to 50 μL 4 mM 4-N,N-dimethylaminoazobenzene-4'-isothiocyanate in acetone, heat at 54° for 1 h, dry under vacuum, dissolve the residue in 100 μL 50% trifluoroacetic acid, heat at 54° for 45 min, dry under vacuum, reconstitute with EtOH:water, inject a 20 μL aliquot. (Purify 4-N,N-dimethylaminoazobenzene-4'-isothiocyanate by dissolving 500 mg in 50 mL boiling acetone, filter (sintered glass), store the filtrate at -20° overnight to obtain 4-N,N-dimethylaminoazobenzene-4'-isothiocyanate as needle-shaped crystals.)

HPLC VARIABLES
Guard column: 20 × 4.6 40 μm Pellicular Packing LC-18
Column: 250 × 4.6 5 μm Supelcosil LC-18
Mobile phase: Gradient. MeCN:35 mM pH 5.1 sodium acetate buffer 39:61 for 8 min, to 53:47 over 4 min, maintain at 53:47 for 28 min, re-equilibrate at initial conditions for 10 min.
Flow rate: 1
Injection volume: 20
Detector: UV 436

CHROMATOGRAM
Retention time: 5 (cysteic acid), 6 (aspartic acid), 7 (carboxymethylcysteine), 5 (glutamic acid), 10.8 (glutamine), 12 (glutamine), 12.8 (asparagine), 13.2 (serine), 14 (threonine), 16.4 (glycine), 17.2 (histidine), 18.8 (tyrosine), 19.6 (alanine), 17.2 (methionine), 18 (tryptophan), 18.8 (valine), 19.6 (proline), 32.4 (phenylalanine), 37.2 (isoleucine), 38.4 (leucine)
Limit of detection: <1 pmole

KEY WORDS
derivatization

REFERENCE
Stocchi,V.; Cucchiarini,L.; Piccoli,G.; Magnani,M. Complete high-performance liquid chromatographic separation of 4-N,N-dimethylaminoazobenzene-4'-thiohydantoin and 4-dimethylaminoazobenzene-4'-sulphonyl chloride amino acids utilizing the same reversed-phase column at room temperature, *J.Chromatogr.*, **1985**, *349*, 77–82.

SAMPLE
Matrix: bulk
Sample preparation: Dissolve 250 nmoles of the amino acid in 500 μL 50 mM pH 10.0 sodium borate buffer, add 500 μL 6 mM 4-N,N-dimethylamino-1-naphthyl isothiocyanate

in dioxane (Caution! Dioxane is a carcinogen!), purge with nitrogen, heat at 40° for 1.5 min, concentrate under a stream of nitrogen, add 500 μL water, add 1 mL hexane, vortex, centrifuge, discard the hexane layer, repeat the hexane wash twice more. Dry the aqueous layer under a stream of nitrogen, reconstitute with 500 μL trifluoroacetic acid:water 50:50, purge with nitrogen, heat at 80° for 10 min, dry under a stream of nitrogen, reconstitute with 1 mL MeCN, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 μm TSK-gel ODS-120A (Toyo Soda, Tokyo)
Mobile phase: Gradient. A was MeCN:50 mM pH 7.0 imidazole nitrate buffer 15:85. B was MeCN:water 60:40. A:B from 100:0 to 0:100 over 25 min.
Flow rate: 1
Injection volume: 10
Detector: F ex 345 em 435 following post-column reaction. The column effluent mixed with MeCN:150 mM NaOH 60:40 pumped at 1 mL/min and the mixture flowed through a 50 cm × 0.5 mm ID stainless steel coil to the detector.

CHROMATOGRAM

Retention time: 12.1, 12.4 (Asp), 13.6 (Glu), 13.6, 14.1 (carboxymethylcysteine), 17.4 (Asn), 17.6, 18.7 (His), 18.0, 18.4 (Ser), 18.5 (Gln), 18.6, 19.0 (Thr), 20.3, 20.9 (Arg), 20.6 (Gly), 21.8, 23.0 (Tyr), 22.7 (Ala), 25.9, 27.2 (Trp), 26.2, 26.6 (Met), 26.7 (Val), 27.1, 31.5 (Lys), 27.8, 28.5 (Phe), 28.7 (Ile), 29.0 (Leu)
Limit of detection: 0.2 pmole

KEY WORDS

derivatization; post-column reaction; stereoisomers give rise to two peaks for some amino acids

REFERENCE

Miyano,H.; Nakajima,T.; Imai,K. Micro-scale sequence analysis from the N-terminus of peptides using the fluorogenic Edman reagent 4-*N,N*-dimethylamino-1-naphthyl isothiocyanate, *Biomed.Chromatogr.*, **1987**, *2*, 139–144.

SAMPLE

Matrix: bulk
Sample preparation: Dissolve 5 mg amino acids in 10 mL MeCN:water:triethylamine 50:50:0.55. Remove a 50 μL aliquot and add it to 50 μL 0.66% 2,3,4,6-tetra-O-benzoyl-β-D-glucopyranosyl isothiocyanate (Fluka) in MeCN, shake mechanically for 30 min, add 10 μL 0.26% ethanolamine in MeCN, shake for 10 min, make up to 1 mL with MeCN, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 25 × 4 (sic) 5 μm LiChrospher 100 RP-18
Mobile phase: MeOH:water:67 mM pH 7.0 phosphate buffer 65:27:8 (A) or 70:25:5 (B) or 80:15:5 (C)
Flow rate: 0.42 (A) or 0.45 (B) or 0.50 (C)
Injection volume: 10
Detector: UV 231

CHROMATOGRAM

Retention time: k' 5.19 (D-proline (B)), k' 5.35 (L-threonine (B)), k' 6.22 (L-tyrosine (B)), k' 6.24 (D-threonine (B)), k' 6.24 (L-2-aminobutyric acid (B)), k' 6.38 (L-phenylglycine (B)), k' 6.41 (L-proline (B)), k' 7.22 (L-valine (B)), k' 7.37 (L-penicillamine (B)), k' 7.41 (D-tyrosine (B)), k' 7.57 (D-2-aminobutyric acid (B)), k' 7.86 (D-phenylglycine (B)), k' 8.08 (L-methionine (B)), k' 9.16 (D-valine (B)), k' 9.27 (L-isoleucine (B)), k' 9.43 (L-tryptophan (B)), k' 9.51 (L-leucine (B)), k' 10.05 (D-penicillamine (B)), k' 10.24 (D-methionine (B)), k' 10.54 (L-phenylalanine (B)), k' 11.94 (L-ornithine (C)), k' 12.03 (D-tryptophan (B)), k' 12.35 (D-isoleucine (B)), k' 12.65 (D-leucine (B)), k' 12.89 (L-norleucine (B)), k' 13.48 (L-

lysine (C)), k' 13.81 (D-phenylalanine (B)), k' 13.90 (D-ornithine (C)), k' 15.32 (D-lysine (C)), k' 16.81 (D-norleucine (B)), k' 16.88 (L-3-aminobutyric acid (A)), k' 18.00 (L-alanine (A)), k' 18.95 (D-3-aminobutyric acid (A)), k' 20.85 (D-alanine (A))

KEY WORDS
derivatization; chiral

REFERENCE
Lobell,M.; Schneider,M.P. 2,3,4,6-Tetra-O-benzoyl-β-D-glucopyranosyl isothiocyanate: an efficient reagent for the determination of enantiomeric purities of amino acids, β-adrenergic blockers and alkyloxiranes by high-performance liquid chromatography using standard reversed-phase columns, *J.Chromatogr.*, **1993**, *633*, 287–294.

SAMPLE
Matrix: bulk
Sample preparation: Dissolve amino acids in 70 μL AccQ.Fluor Borate Buffer (Waters), add 20 μL AccQ.Fluor Reagent (10 mM 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate in MeCN (Waters)), vortex, let stand for 1 h at room temperature, heat at 55° for 10 min, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm Chiradex (immobilized β-cyclodextrin) (Merck)
Mobile phase: MeOH:100 mM pH 6.5 ammonium acetate buffer containing 0.1% triethylamine 50:50
Column temperature: 20 ± 0.1
Flow rate: 0.5
Injection volume: 20
Detector: UV 254 or F ex 250 em 395

CHROMATOGRAM
Retention time: k' 2.45 (second enantiomer, α = 1.08 (Ala)), k' 2.15 (second enantiomer, α = 1.00 (Val)), k' 4.89 (second enantiomer, α = 1.39 (Leu)), k' 3.16 (second enantiomer, α = 1.13 (Ile)), k' 2.51 (second enantiomer, α = 1.10 (Pro)), k' 2.90 (second enantiomer, α = 1.08 (Met)), k' 2.40 (second enantiomer, α = 1.08 (Cys)), k' 2.50 (second enantiomer, α = 1.07 (Ser)), k' 2.29 (second enantiomer, α = 1.07 (Thr)), k' 4.86 (second enantiomer, α = 1.16 (Lys)), k' 1.44 (second enantiomer, α = 1.13 (Arg)), k' 2.46 (second enantiomer, α = 1.11 (Asn)), k' 2.05 (second enantiomer, α = 1.06 (Gln)), k' 16.64 (second enantiomer, α = 1.20 (Asp)), k' 10.08 (second enantiomer, α = 1.00 (Glu)), k' 7.06 (second enantiomer, α = 1.13 (Phe)), k' 4.82 (second enantiomer, α = 1.04 (Trp)), k' 2.50 (second enantiomer, α = 1.06 (His)), k' 5.19 (second enantiomer, α = 1.25 (Tyr))

KEY WORDS
chiral; derivatization; α = k' (second enantiomer)/k' (first enantiomer); detailed comparison with other derivatizing reagents

REFERENCE
Rizzi,A.M.; Cladrowa-Runge,S.; Jonsson,H.; Osla,S. Enantiomeric resolution of derivatized DL-amino acids by high-performance liquid chromatography using a β-cyclodextrin chiral stationary phase: A comparison between derivatization labels, *J.Chromatogr.A*, **1995**, *710*, 287–295.

SAMPLE
Matrix: bulk
Sample preparation: Dissolve 5 mg amino acids in 460 μL 100 mM boric acid adjusted to pH 9 with NaOH, add 300 μL 100 mM dansyl chloride in acetone, let stand in dark for 2 h, evaporate to dryness under reduced pressure, reconstitute with 500 μL acetone:1 M HCl 95:5, centrifuge for 5 min, evaporate the liquid to dryness under reduced pressure, reconstitute with mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4 5 μm Chiradex (immobilized β-cyclodextrin) (Merck)
Mobile phase: MeOH:100 mM pH 5.5 ammonium acetate buffer containing 0.1% triethylamine 70:30
Column temperature: 20 ± 0.1
Flow rate: 0.5
Injection volume: 20
Detector: UV 254, F ex 325 em 350

CHROMATOGRAM

Retention time: k' 2.52 (second enantiomer, α = 1.08 (Ala)), k' 2.03 (second enantiomer, α = 1.22 (Val)), k' 2.54 (second enantiomer, α = 1.75 (Leu)), k' 1.68 (second enantiomer, α = 1.32 (Ile)), k' 1.40 (second enantiomer, α = 1.10 (Pro)), k' 1.33 (second enantiomer, α = 1.24 (Met)), k' 2.99 (second enantiomer, α = 1.15 (Cys)), k' 1.37 (second enantiomer, α = 1.16 (Ser)), k' 1.46 (second enantiomer, α = 1.29 (Thr)), k' 1.22 (second enantiomer, α = 1.10 (Lys)), k' 0.84 (second enantiomer, α = 1.16 (Arg)), k' 1.68 (second enantiomer, α = 1.11 (Asn)), k' 1.38 (second enantiomer, α = 1.14 (Gln)), k' 18.03 (second enantiomer, α = 1.13 (Asp)), k' 9.17 (second enantiomer, α = 1.15 (Glu)), k' 2.42 (second enantiomer, α = 1.43 (Phe)), k' 1.63 (second enantiomer, α = 1.00 (Trp)), k' 1.66 (second enantiomer, α = 1.07 (His)), k' 2.67 (second enantiomer, α = 1.25 (Tyr))

KEY WORDS

chiral; derivatization; α = k' (second enantiomer)/k' (first enantiomer); detailed comparison with other derivatizing reagents

REFERENCE

Rizzi,A.M.; Cladrowa-Runge,S.; Jonsson,H.; Osla,S. Enantiomeric resolution of derivatized DL-amino acids by high-performance liquid chromatography using a β-cyclodextrin chiral stationary phase: A comparison between derivatization labels, *J.Chromatogr.A*, **1995**, *710*, 287–295.

SAMPLE

Matrix: bulk
Sample preparation: Dissolve 1 mg amino acid in 250 μL water and 250 μL 40 mM pH 7.7 borate buffer, add 400 μL MeCN, add 3 mg reagent, vortex, let stand for about 30 min, extract with 3 mL ethyl acetate, inject an aliquot of the organic layer. (Synthesize the reagent, fluorenylmethoxycarbonyl glycyl chloride (FMOC-glycyl-Cl), as follows. Dissolve 1.0 g fluorenylmethoxycarbonyl glycine (FMOC glycine) in 15 mL dichloromethane, 3.4 mL thionyl chloride, reflux for 4 h, evaporate to dryness under reduced pressure, dissolve the residue in 1 mL dichloromethane, add 10 mL hexane. Filter the precipitate and dry it under vacuum at room temperature for 2 h to obtain fluorenylmethoxycarbonyl glycyl chloride.)

HPLC VARIABLES

Column: 250 × 4.6 5 μm gamma-cyclodextrin Cyclobond II
Mobile phase: MeCN:triethylamine:acetic acid 100:1.2:0.3
Flow rate: 1
Detector: UV 265

CHROMATOGRAM

Retention time: k' 2.9 (methionine, α = 1.10), k' 3.0 (valine, α = 1.00), k' 3.1 (norleucine, α = 1.07), k' 3.1 (O-methyltyrosine, α = 1.10), k' 3.3 (norvaline, α = 1.09), k' 3.4 (phenylalanine, α = 1.11), k' 3.4 (alanine, α = 1.07), k' 3.6 (leucine, α = 1.15), k' 4.1 (homophenylalanine, α = 1.11), k' 8.3 (asparagine, α = 1.17), k' 8.66 (glutamine, α = 1.26), k' 12.1 (tryptophan, α = 1.29), k' 13.0 (aspartic acid, α = 1.06), k' 37.4 (glutamic acid, α = 1.21) (k' is the capacity factor of the first eluted enantiomer which is L except for aspartic acid, phenylalanine, and tryptophan.)

KEY WORDS

derivatization; chiral; comparison with other derivatizing reagents; details of chromatography with other mobile phases and with a β-cyclodextrin column are also given in the paper

REFERENCE

Tang,Y.; Zukowski,J.; Armstrong,D.W. Investigation on enantiomeric separations of fluorenylmethoxycarbonyl amino acids and peptides by high-performance liquid chromatography using native cyclodextrins as chiral stationary phases, *J.Chromatogr.A*, **1996**, *743*, 261–271.

SAMPLE

Matrix: bulk

Sample preparation: Treat 0.2-10 μmoles amino acids with 50-100 μL MeOH:thionyl chloride 95:5 at 60° for 2 h, evaporate to dryness, reconstitute with 35 μL 28% diisopropylethylamine in DMF, shake at room temperature for 10 min, add 25 μL 200-1000 mM N-α-(9-fluorenylmethyloxycarbonyl)leucine-N-carboxyanhydride in DMF, mix, let stand at room temperature for 10 min, add 200 μL 250 mM pH 8 sodium glycinate, mix, let stand for 5 min, add 300 μL chloroform, extract, dilute 10000-50000-fold with n-hexane, inject a 20 μL aliquot. (Synthesis of N-α-(9-fluorenylmethyloxycarbonyl)leucine-N-carboxyanhydride is as follows. Dry all solvents over 4 Å molecular sieve. Stir 20 mmoles L-leucine in 67 mL THF under nitrogen at 2° in an ice bath, add 22 mmoles 9-fluorenylmethyl chloroformate at once, slowly add 29 mmoles dry 4-methylmorpholine (N-methylmorpholine), stir at 2-5° for 2 h, slowly add 4 M HCl in dioxane (Caution! Dioxane is a carcinogen!) until the pH of a sample diluted with water reaches 4-5, filter, wash the solid with dry THF, concentrate the filtrate under reduced pressure, dissolve the resulting oil in the minimum volume of dry diisopropyl ether (Caution! Diisopropyl ether readily forms explosive peroxides!), add dry hexane until the solution just turns cloudy, let stand at -20° overnight, filter. Wash the solid with dry hexane and dry it under vacuum to obtain N-α-(9-fluorenylmethyloxycarbonyl)leucine-N-carboxyanhydride (mp 118-120°; $[\alpha]^{25}_{D}$ = +38.0°) (J. Am. Chem. Soc. 1990, 112, 7414).)

HPLC VARIABLES

Guard column: 15 × 3.2 5 μm Kromasil silica
Column: 250 × 4.6 5 μm Kromasil silica
Mobile phase: n-Hexane:isopropanol 98:2 (A) or 97:3 (B) or 95:5 (C) or 90:10 (D)
Flow rate: 0.8
Injection volume: 20
Detector: F ex 263 em 313

CHROMATOGRAM

Retention time: k' 1.35 (L-tyrosine (D)), k' 1.42 (L-tryptophan (D)), k' 1.66 (D-tyrosine (D)), k' 1.70 (L-threonine (D)), k' 1.72 (D-tryptophan (D)), k' 1.94 (D-threonine (D)), k' 1.97 (L-lysine (C)), k' 1.98 (L-leucine (A)), k' 2.11 (L-isoleucine (A)), k' 2.20 (L-valine (A)), k' 2.24 (L-2-aminohexanoic acid (A)), k' 2.37 (D-leucine (A)), k' 2.50 (L-β-(1-naphthyl)alanine (UV detection) (A)), k' 2.55 (L-phenylalanine (A)), k' 2.58 (L-2-amino-4-phenylbutyric acid (A)), k' 2.60 (D-isoleucine (A)), k' 2.60 (D-valine (A)), k' 2.60 (L-2-aminopentanoic acid (A)), k' 2.75 (D-2-aminohexanoic acid (A)), k' 2.87 (L-β-(2-naphthyl)alanine (UV detection) (A)), k' 2.88 (L-α-aminophenylacetic acid (A)), k' 2.94 (L-β-(2-thienyl)alanine (A)), k' 2.98 (L-β-(p-chlorophenyl)alanine (A)), k' 3.03 (D-lysine (C)), k' 3.12 (L-β-(3,4-dichlorophenyl)alanine (A)), k' 3.18 (D-2-aminopentanoic acid (A)), k' 3.27 (L-serine (C)), k' 3.35 (L-methionine (A)), k' 3.35 (D-2-amino-4-phenylbutyric acid (A)), k' 3.38 (L-1,2,3,4-tetrahydro-3-isoquinolinecarboxylic acid), k' 3.42 (D-α-aminophenylacetic acid (A)), k' 3.51 (D-β-(1-naphthyl)alanine (UV detection) (A)), k' 3.59 (D-serine (C)), k' 3.61 (L-α-aminobutyric acid (A)), k' 4.11 (D-1,2,3,4-tetrahydro-3-isoquinolinecarboxylic acid (A)), k' 4.14 (D-β-(2-thienyl)alanine (A)), k' 4.34 (D-α-aminobutyric acid (A)), k' 4.42 (D-β-(2-naphthyl)alanine (UV detection) (A)), k' 4.71 (D-β-(p-chlorophenyl)alanine (A)), k' 4.88 (D-β-(3,4-dichlorophenyl)alanine (A)), k' 4.91 (D-methionine (A)), k' 4.91 (D-phenylalanine (A)), k' 5.04 (L-glutamic acid (B)), k' 5.37 (L-alanine (A)), k' 5.97 (D-alanine (A)), k' 6.24 (L-aspartic acid (B)), k' 7.35 (D-aspartic acid (B)), k' 7.8 (D-glutamic acid (B))

KEY WORDS
derivatization; normal phase; the use of related derivatization reagents is discussed; chiral

REFERENCE
Pugniere,M.; Mattras,H.; Castro,B.; Previero,A. Adsorption liquid chromatography on silica for the chiral separation of amino acids and asymmetric amines derivatized with optically active N-α-9-fluorenylmethyloxyxarbonyl-amino acid-N-carboxyanhydrides, *J.Chromatogr.A*, **1997**, *767*, 69–75.

SAMPLE
Matrix: cell suspensions
Sample preparation: Centrifuge 350 μL cell suspensions at 1470 g for 1 min, add 20 μL 16% trichloroacetic acid to the supernatant, freeze. Thaw the supernatant and adjust its pH to 7.0 with 1 M NaOH, remove a 300 μL aliquot and make up to 400 μL with water, add 100 μL 1 M pH 6.2 boric acid, add 40 μL 10 μM dihydrokainic acid in MeCN:water 10:90, vortex for 10 s, add 500 μL 15 mM 9-fluorenylmethyl chloroformate, mix for 45 s, add 1 mL ethyl acetate, mix for 10 s, centrifuge at 10500 g for 2 min, repeat the ethyl acetate wash, inject an aliquot of the aqueous layer.

HPLC VARIABLES
Column: 250 × 4.6 C18 (Phenomenex)
Mobile phase: Gradient. A was 0.1% trifluoroacetic acid in MeCN. B was 0.1% trifluoroacetic acid in water. A:B from 30:70 to 50:50 over 15 min, to 100:0 over 2 min, maintain at 100:0 for 5 min, return to initial conditions over 2 min, re-equilibrate for 12 min (cf. Int. J. Environ. Anal. Chem. 1990, 38, 351).
Column temperature: 55
Flow rate: 1
Injection volume: 25
Detector: F ex 264 em 313

CHROMATOGRAM
Retention time: 3.5 (taurine), 11 (glutamine), 12.5 (glutamate), 13 (aspartate)
Internal standard: dihydrokainic acid (21)

KEY WORDS
derivatization

REFERENCE
Brown,J.A.; Nijjar,M.S. The release of glutamate and aspartate from rat brain synaptosomes in response to domoic acid (amnesic shellfish toxin) and kainic acid, *Mol.Cell.Biochem.*, **1995**, *151*, 49–54.

SAMPLE
Matrix: cheese
Sample preparation: Homogenize (Ultra-Turrax T25) 16 g cheese with 30 mL water at room temperature for 2 min, centrifuge at 4° at 8650 g for 20 min, remove the water and fat layers. Homogenize the pellet in 30 mL water, centrifuge, remove the water and fat layers, repeat this process. Combine the fat layers and homogenize them with 20 mL water, centrifuge, remove the water layer. Combine all the water layers, filter (Whatman No. 42 paper) at 4°, filter (0.45 μm) at 4°, purify on a 200 × 4 Sephadex G-25 column with water at 84 mL/min with detection at UV 206, collect 84 mL fractions. Evaporate a 200 μL aliquot to dryness under reduced pressure, reconstitute with 200 μL 500 mM pH 7.8 borate buffer, add 200 μL 5.8 mM 9-fluorenylmethyl chloroformate in acetone, vortex for 45 s, wash with 400 μL pentane:ethyl acetate 80:20, inject an aliquot of the aqueous phase.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Nucleosil C18

Mobile phase: Gradient. A was MeCN:100 mM pH 3.8 ammonium acetate buffer 20:80. B was MeCN:100 mM pH 4.2 ammonium acetate buffer 80:20. A:B 70:30 for 10 min, to 20:80 over 50 min, to 0:100 (step gradient), maintain at 0:100 for 10 min.
Column temperature: 40
Flow rate: 1
Detector: UV 214

CHROMATOGRAM

Retention time: 20 (arginine), 22.5 (serine, aspartic acid), 25 (glutamic acid), 27 (threonine), 30 (glycine), 35 (alanine), 40 (proline), 44 (valine), 46 (methionine), 51 (phenylalanine), 52.5 (isoleucine, leucine), 65 (histidine), 67.5 (lysine)

OTHER SUBSTANCES

Also analyzed: dipeptides, tripeptides

KEY WORDS

derivatization

REFERENCE

Roturier,J.M.; Le Bars,D.; Gripon,J.C. Separation and identification of hydrophilic peptides in dairy products using FMOC derivatization, *J.Chromatogr.A*, **1995**, *696*, 209–217.

SAMPLE

Matrix: chitin, protein, soil
Sample preparation: Hydrolyse 2.5 g sample with 90 mL boiling 6 M HCl for 6 h, filter, dilute filtrate to 100 mL, add 2 mL L-norleucine solution. Evaporate a 10 μL aliquot to dryness under reduced pressure, reconstitute with 20 μL 20 mM HCl, add 60 μL 200 mM pH 8.8 borate buffer, add 20 μL 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (Waters) in MeCN, heat at 40° for 5 min, inject an aliquot.

HPLC VARIABLES

Column: 150 × 3.9 4 μm AccQ-Tag C18 (Waters)
Mobile phase: Gradient. MeCN:water:buffer from 0:0:100 to 1:0:99 over 0.5 min, to 5:0:95 over 17.5 min, to 9:0:91 over 1 min, to 17:0:83 over 10.5 min, to 60:40:0 over 3.5 min, return to initial conditions over 22 min. (Buffer was 140 mM sodium acetate containing 17 mM triethylamine, adjusted to pH 5.05 with phosphoric acid.)
Column temperature: 37
Flow rate: 1
Injection volume: 5
Detector: F ex 250 em 395

CHROMATOGRAM

Retention time: 7.8 (β-Galactosamine), 18.9 (α-Galactosamine), 9.1 (β-Glucosamine), 15.9 (α-Glucosamine), 17.0 (Asp), 19.3 (Ser), 19.6 (Glu), 21.3 (Gly), 22.2 (His), 23.3 (ammonia), 25.8 (Arg), 26.2 (Thr), 26.9 (Ala), 28.2 (Pro), 29.3 (α-aminobutyric acid), 31.3 (Tyr), 32.4 (Val), 32.8 (Met), 35.7 (Lys), 36.1 (Ile), 36.5 (Leu), 38.3 (Phe)
Internal standard: L-norleucine (37.4)
Limit of detection: 49-780 fmole

KEY WORDS

derivatization

REFERENCE

Díaz,J.; Lliberia,J.L.; Comellas,L.; Broto-Puig,F. Amino acid and amino sugar determination by derivatization with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate followed by high-performance liquid chromatography and fluorescence detection, *J.Chromatogr.A*, **1996**, *719*, 171–179.

SAMPLE

Matrix: collagen

Sample preparation: Hydrolyze protein with 6 M HCl under nitrogen at 108° for 24 h, evaporate to dryness under reduced pressure, reconstitute with 100 mM sodium bicarbonate to give a hydrolysate concentration of 1 μg/mL. Remove a 5 μL aliquot and dilute it to 200 μL with 100 mM sodium bicarbonate, add 200 μL 4 mM fluorenylmethyl chloroformate in dry acetone, shake quickly, let stand at room temperature for 10 min, wash twice with 600 μL portions of pentane:ethyl acetate 90:10, inject a 20 μL aliquot of the lower aqueous phase.

HPLC VARIABLES

Column: 150 × 4.6 MicroPak ODS-80TM

Mobile phase: Gradient. A was buffer. B was MeOH:buffer adjusted to pH 4.5 with phosphoric acid 20:80. C was MeCN. A:B:C 72.5:0:27.5 for 4.1 min, to 60:0:40 over 11.5 min, to 0:64:36 over 0.1 min, to 0:62:38 over 6.3 min, to 0:30:70 over 7 min, to 0:25:75 over 5 min, return to initial conditions, re-equilibrate for 11 min. (Buffer was 20 mM sodium citrate containing 5 mM tetramethylammonium chloride, pH 2.85.)

Column temperature: 30

Flow rate: 1.4

Injection volume: 20

Detector: F ex 254 em 340

CHROMATOGRAM

Retention time: 3.50 (histidine (mono-derivative)), 4.24 (cysteic acid), 4.78 (arginine), 7.60 (methionine sulfoxide), 8.92 (4-hydroxyproline), 9.80 (serine), 10.22 (3-hydroxyproline, homoserine), 10.80 (aspartic acid), 11.68 (glutamic acid), 12.48 (threonine), 13.05 (S-carboxymethylcysteine), 13.96 (glycine), 16.21 (ammonia), 16.60 (alanine), 17.48 (tyrosine (mono-derivative)), 18.30 (proline), 19.90 (methionine), 21.00 (valine), 23.27 (phenylalanine), 23.66 (isoleucine), 24.02 (leucine), 26.90 (cystine (bis-derivative)), 28.17 (hydroxylysine (bis-derivative), 28.81 (histidine (bis-derivative)), 30.00 (lysine (bis-derivative)), 31.85 (tyrosine (bis-derivative))

Limit of quantitation: 1 pmole

KEY WORDS

derivatization

REFERENCE

Miller,E.J.; Narkates,A.J.; Niemann,M.A. Amino acid analysis of collagen hydrolysates by reverse-phase high-performance liquid chromatography of 9-fluorenylmethyl chloroformate derivatives, *Anal.Biochem.*, **1990**, *190*, 92–97.

SAMPLE

Matrix: contact lenses

Sample preparation: Hydrolyze contact lens with 200 μL concentrated HCl at 145° for 1 h or with 200 μL 6 M HCl at 105° for 20 h, remove a 50-150 μL aliquot and evaporate it to dryness under reduced pressure, add 20 μL EtOH:water:triethylamine 40:40:20, evaporate to dryness under reduced pressure, add 30 μL EtOH:water:triethylamine:phenylisothiocyanate 70:10:10:10, let stand at room temperature for 25 min, reconstitute with 30-200 μL buffer, inject a 10 μL aliquot. (Buffer was MeCN:5 mM Na_2HPO_4 adjusted to pH 6.8 with 2% phosphoric acid.)

HPLC VARIABLES

Column: 150 × 4.6 Ultrasphere ODS C18

Mobile phase: Gradient. A was 700 μL triethylamine in 1 L 100 mM sodium acetate, adjusted to pH 5.5 with glacial acetic acid. B was MeCN:A:water 315:250:185. A:B 90:10 for 2 min, to 62:38 over 6 min, to 55:45 over 0.5 min, to 40:60 over 7 min, to 10:90 over 3 min, to 0:100 over 1 min, return to initial conditions over 8 min, re-equilibrate for 6 min. (Mobile phase A maintained at 60°, mobile phase B maintained at 40°.)

Column temperature: 43
Flow rate: 1
Injection volume: 10
Detector: UV 254

KEY WORDS
derivatization

REFERENCE
Yan,G.; Nyquist,G.; Caldwell,K.D.; Payor,R.; McCraw,E.C. Quantitation of total protein deposits on contact lenses by means of amino acid analysis, *Invest.Ophthalmol.Vis.Sci.*, **1993**, *34*, 1804–1813.

SAMPLE
Matrix: dialysate, tissue
Sample preparation: Homogenize (Kontes micro-ultrasonic cell disrupter) rat brain with 100 μL 50 mM ice-cold perchloric acid and 10 ng homoserine for 5 s, centrifuge at 4° at 13000 g for 5 min, filter (0.2 μm) the supernatant. Mix 25 μL of the filtrate from the tissue or dialysate (Ringer's) with 50 (tissue) or 12.5 (dialysate) μL working reagent, let stand for 2 min, inject an aliquot. (Prepare the reagent stock solution by dissolving 27 mg o-phthalaldehyde in 1 mL MeOH, add 5 μL β-mercaptoethanol, add 9 mL 100 mM pH 9.3 sodium tetraborate, discard after 5 days. Prepare the working reagent by diluting 1 mL stock solution with 3 mL 100 mM sodium tetraborate, let stand for 24 h before use.)

HPLC VARIABLES
Column: 80 × 4.6 3 μm C18 HR-80 (ESA)
Mobile phase: MeOH:water 28:72 containing 100 mM Na_2HPO_4 and 0.13 mM disodium EDTA adjusted to pH 6.00 (tissue) or pH 6.40 (dialysate) with phosphoric acid. (Prepare by dissolving 14.2 g Na_2HPO_4 and 50 mg disodium EDTA in 720 mL water, add 280 mL MeOH, adjust pH. Recycle mobile phase.)
Flow rate: 1.2
Injection volume: 20
Detector: E, ESA Model 5100A coulometric, model 5011 dual electrode analytical cell preceded by a 0.2 μm carbon filter at -0.4 V and +0.6 V

CHROMATOGRAM
Retention time: 1.5 (Asp), 2 (Glu), 3 (Ser), 4 (Gln), 5.5 (Gly, Thr), 7 (Phenylethanolamine), 9 (Taurine), 10.5 (Ala), 14 (Tyr), 15 (GABA)
Internal standard: homoserine (3.5)
Limit of detection: 100-200 pg

KEY WORDS
rat; brain; derivatization

REFERENCE
Donzanti,B.A.; Yamamoto,B.K. An improved and rapid HPLC-EC method for the isocratic separation of amino acid neurotransmitters from brain tissue and microdialysis perfusates, *Life Sci.*, **1988**, *43*, 913–922.

SAMPLE
Matrix: enzyme incubations
Sample preparation: 1 mL Enzyme incubation + 11 mL 155 μM DL-α-aminobutyric acid in buffer, mix, add a 2 mL aliquot to an 8 mL column of Sephadex G-25 (Pharmacia PD-10), elute with buffer, discard the first 6 mL eluate, collect the next 4 mL eluate. Add 3.2 μL diethyl ethoxymethylenemalonate to this fraction, shake at 50° for 50 min, inject a 15 μL aliquot. (Buffer was 1 M pH 9 sodium borate buffer containing 0.02% sodium azide.)

HPLC VARIABLES
Column: 300 × 3.9 4 μm Nova-Pak C18

Mobile phase: Gradient. MeCN:25 mM pH 6 sodium acetate containing 0.02% sodium azide from 9:91 to 14:86 over 3 min, maintain at 14:86 for 10 min, to 31:69 over 17 min, maintain at 31:69 for 20 min. (Caution! Sodium azide is highly toxic! Do not discharge to the plumbing system!)
Flow rate: 0.9
Injection volume: 15
Detector: UV 280

CHROMATOGRAM

Retention time: 17.7 (N-epsilon-(2-propenal)lysine), 35 (lysine), 44.8 (lysine dipeptide)
Internal standard: DL-α-aminobutyric acid (19)
Limit of detection: <1 pmole

KEY WORDS

derivatization

REFERENCE

Girón,J.; Alaiz,M.; Vioque,E. High-performance liquid chromatographic determination of *N*-epsilon-(2-propenal)lysine in biological samples after derivatization with diethylethoxymethylenemalonate, *Anal.Biochem.*, **1992**, *206*, 155–160.

SAMPLE

Matrix: feces, insects, plants
Sample preparation: Grind black cherry leaves, insect larvae, or feces to pass 40 mesh. Weigh out an amount containing 0.2-2 mg protein, add 2 mL 4 M methanesulfonic acid in water containing 0.2% 3-(2-aminoethyl)indole, freeze in dry ice-acetone, evacuate to 50 μTorr, flush with nitrogen, evacuate to 50 μTorr, flush with nitrogen, evacuate to 50 μTorr, hydrolyze at 115° for 22 h, add c-allylglycine, buffer with 100 mM pH 8.0 sodium borate, adjust to pH 8.0 with NaOH, make up to 12 mL, centrifuge. Remove a 1 mL aliquot and add it to 1 mL 6 mM 9-fluorenylmethylchloroformate in acetone, let stand for 15 min at room temperature, extract twice with 2 mL portions of n-pentane, filter (0.45 μm), discard the first few drops, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 end-capped ODS-80TM Aminotag (Varian)
Mobile phase: Gradient. A was MeCN:15 mM citric acid + 10 mM tetramethylammonium chloride adjusted to pH 1.85 with NaOH 27:73. B was MeCN:THF:15 mM citric acid + 10 mM tetramethylammonium chloride adjusted to pH 4.50 with NaOH 35:5:60. C was MeCN:THF:15 mM citric acid + 10 mM tetramethylammonium chloride adjusted to pH 4.50 with NaOH 62:13:25. A:B:C from 100:0:0 to 50:50:0 over 3 min, to 0:100:0 over 14 min (Waters concave gradient 7), to 0:96:4 over 6 min (Waters concave gradient 7), to 0:85:15 over 4 min, to 0:50:50 over 4 min, to 0:35:65 over 4 min, to 0:0:100 over 5 min. (Linear gradients except where shown).
Column temperature: 30
Flow rate: 1.4
Injection volume: 20
Detector: UV 264

CHROMATOGRAM

Retention time: 7.5 (His), 9.5 (Arg), 11.5 (Asn), 11.5 (Gln), 13 (Ser), 14 (Asp), 15 (Glu), 15.5 (Thr), 17 (Gly), 20.5 (Ala), 22.5 (Tyr), 23 (Pro), 26 (Met), 27 (Val), 29.5 (Phe), 30.5 (Trp), 31.5 (Ile), 32 (Leu), 33 (cystine), 35.5 (di-His), 36.5 (Cys), 37.5 (Lys), 40 (di-Tyr)
Internal standard: c-allylglycine (25)
Limit of quantitation: 30000 nM

KEY WORDS

derivatization; protein; hydrolysis; plants; insects; feces; cherry; leaves; insect; larvae; feces; ASN and GLN co-elute

REFERENCE
Malmer,M.F.; Schroeder,L.A. Amino acid analysis by high-performance liquid chromatography with methanesulfonic acid hydrolysis and 9-fluorenylmethylchloroformate derivatization, *J.Chromatogr.*, **1990**, *514*, 227–239.

SAMPLE
Matrix: feed
Sample preparation: Heat 500 mg feed and 2 mL 6 M HCl containing 1% phenol at 105° for 24 h, neutralize with 100 mM NaOH, make up to 100 mL, filter (0.22 μm nylon). Mix 150 μL hydrolysate with 150 μL 30 mM sodium 1,2-naphthoquinone-4-sulfonate in 100 mM HCl and 150 μL 50 mM sodium borate containing 90 mM NaOH, heat at 65° for 5 min, add 60 μL 250 mM HCl, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 3 μm Spherisorb ODS 2
Mobile phase: Gradient. A was buffer. B was MeCN:buffer 50:50. A:B 100:0 for 15 min, to 94:6 over 5 min, maintain at 94:6 for 10 min, to 86:14 over 1 min, to 83:17 over 14 min, to 73:27 over 1 min, to 71:29 over 14 min, to 0:100 over 2 min, maintain at 0:100 for 8 min, return to initial conditions over 2 min, re-equilibrate for 3 min. (Buffer was 50 mM acetic acid containing 50 mM sodium acetate, pH 4.75.)
Column temperature: 50
Flow rate: 0.8
Injection volume: 50
Detector: UV 305

CHROMATOGRAM
Retention time: 10 (Cys), 12 (Asp), 14.5 (Ser), 21 (Gly), 22 (Glu), 24 (His), 26 (Thr), 29.5 (Pro), 30.5 (Arg), 33.5 (Ala), 41 (Tyr), 45 (Orn), 47.5 (Val), 48 (Met), 42.5 (Lys), 43.5 (Ile), 46 (Leu), 47 (Phe)
Limit of detection: 40-100 pmole

KEY WORDS
derivatization

REFERENCE
Saurina,J.; Hernńdez-Cassou,S. Chromatographic determination of amino acids by pre-column derivatization using 1,2-naphthoquinone-4-sulfonate as reagent, *J.Chromatogr.A*, **1996**, *740*, 21–30.

SAMPLE
Matrix: feed, food
Sample preparation: Add 2 mL chilled performic acid to 50-70 mg sample, let stand at 0° for 16 h, add 300 μL 48% HBr, let stand at 0° for 15 min, evaporate to dryness under reduced pressure at ≤60°, add 10 mL 6 M HCl, freeze in liquid nitrogen, seal tube under vacuum, heat at 110 ± 2° for 22 h, cool to room temperature, filter. Remove a 1-2 mL aliquot and evaporate it to dryness under reduced pressure at ≤50°, reconstitute with 2.5 mM IS solution and water so that the amino acid concentration is <13 mM and the IS concentration is 250 μM. Remove a 10 μL aliquot and add it to 70 μL 200 mM pH 8.8 borate buffer, vortex for 10 s, add 20 μL 3 mg/mL 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (Waters) in MeCN, vortex immediately, heat at 50° for 10 min, inject a 4 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 4 μm AccQ-Tag C18 (Waters)
Mobile phase: Gradient. A was 140 mM sodium acetate containing 17 mM triethylamine and 100 mg/L sodium azide (Caution! Sodium azide is carcinogenic and toxic! Do not discharge to the plumbing system!), pH adjusted to 4.95 with phosphoric acid. B MeCN: water:acetone 60:40:0.01. A:B from 100:0 to 92:8 over 17 min, to 83:17 over 4 min, to 73:

27 over 11 min, to 50:50 over 2 min, maintain at 50:50 for 1 min, to 0:100 over 2 min, return to initial conditions over 1 min, re-equilibrate for 7 min.
Column temperature: 47
Flow rate: 1
Injection volume: 4
Detector: UV 248

CHROMATOGRAM

Retention time: 7.5 (cysteic acid), 12.5 (Asp), 13.5 (Ser), 14.5 (Glu), 15 (Gly), 15.5 (His), 19 (Arg), 19.5 (Thr), 20.7 (methionine sulfone), 21 (Ala), 24 (Pro), 28 (Val), 32 (Lys), 33 (Ile), 33.5 (Leu), 34.5 (Phe)
Internal standard: α-aminobutyric acid (25)

KEY WORDS

derivatization; corn; shrimp

REFERENCE

Liu,H.J.; Chang,B.Y.; Yan,H.W.; Yu,F.H.; Liu,X.X. Determination of amino acids in food and feed by derivatization with 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate and reversed-phase liquid chromatographic separation, *J.AOAC Int.*, **1995**, *78*, 736–744.

SAMPLE

Matrix: fermentation solutions
Sample preparation: Wash 7.5 g broth on a 150 μm nylon mesh with ice-cold 0.8% NaCl, float cells in saline, wash cells with ice water, cool cells in ice, add 5 mL water, add 500 μL 1 M 4-morpholinepropanesulfonic acid buffer, sonicate for 15 s, shake for 30 s, sonicate for 15 s, filter 0.45 μm, filter (10 000 MW cutoff filter) while centrifuging. Add a 200 μL aliquot of the filtrate to a 15 × 8 column of quaternary ion-exchange resin, wash with 10 mL water, elute with 10 mL 1 M acetic acid, freeze dry, reconstitute with 200 μL water. Mix 20 μL solution with 5 μL reagent, let stand for 2-3 min, add 475 μL 50 mM pH 5.2 sodium acetate (Anal.Biochem. 1984, 137, 405), filter (0.45 μm), inject an aliquot of the filtrate. (Prepare reagent by dissolving 4 mg o-phthaldialdehyde in 300 μL MeOH, add 250 μL 400 mM pH 8.0 borate buffer, add 390 μL water, add 60 μL 1 M N-acetyl-L-cysteine (adjusted to pH 5.0-6.0 with NaOH). Store at 4°, discard after 3 weeks (Anal.Biochem. 1984, 137, 405).)

HPLC VARIABLES

Column: 150 × 5 3 μm Hypersil ODS
Mobile phase: Gradient. A was 50 mM pH 5.9 sodium acetate. B was MeOH:50 mM pH 5.9 sodium acetate 80:20. A:B from 100:0 to 0:100 over 50 min (?).
Flow rate: 1
Detector: F ex 330-375 em 418 (cut-off filter)

CHROMATOGRAM

Retention time: 3.5 (glutathione), 4 (L-Asp), 4.5 (L-Glu), 5.8 (L-Ser), 9.5 (L-α-aminoadipic acid), 10.5 (D-α-aminoadipic acid), 11 (L-His), 11.5 (L-Thr), 12 (Gly), 19 (L-Arg), 22.5 (A-Ala), 27 (cephalosporin C), 28 (L-Tyr), 28 (penicillin N), 28.5 (isopenicillin N), 32.5 (L-Val), 34.5 (L-Met), 35 (D-Val), 39 (L-Phe), 40 (L-Ile), 47.5 (L-Leu), 49 (L-Lys)

OTHER SUBSTANCES

Noninterfering: L-cysteine

KEY WORDS

SPE; derivatization; chiral

REFERENCE

Usher,J.J.; Lewis,M.; Hughes,D.W. Determination by high-performance liquid chromatography of some compounds involved in the biosynthesis of penicillin and cephalosporin, *Anal.Biochem.*, **1985**, *149*, 105–110.

SAMPLE

Matrix: fermentation solutions

Sample preparation: Centrifuge 1 mL fermentation medium at 15000 g for 3 min, dilute the supernatant 10-100-fold with water, inject an aliquot.

HPLC VARIABLES

Column: 150 × 3.3 5 μm Separon C18 glass column (Tessek, Prague)

Mobile phase: MeOH:water 25:75 containing 1 mM copper sulfate

Column temperature: 45

Flow rate: 0.5

Injection volume: 1

Detector: UV 245

CHROMATOGRAM

Retention time: 0.5 (alanine), 0.6 (α-aminobutyric acid), 0.9 (valine), 1.65 (leucine), 3.5 (homoleucine)

KEY WORDS

derivatization; complexation

REFERENCE

Polanuer,B.M.; Ivanov,S.V. High-performance liquid chromatography of amino acids in copper(II) complex form: application to valine fermentation samples, *J.Chromatogr.A*, **1996**, *722*, 311–315.

SAMPLE

Matrix: food

Sample preparation: Homogenize (Sorvall) 5 g food with 50 mL 600 mM perchloric acid, centrifuge at 3500 rpm for 20 min, filter (0.45 μm) the supernatant, adjust the pH of the filtrate to 7.0 ± 0.2 with 30% KOH, place in the fridge for 5 min. Evaporate a 1 mL extract to dryness under reduced pressure at 37°, add 20 μL reagent, mix. Let stand at room temperature for 20 min, evaporate to dryness under reduced pressure at 37°, reconstitute with 200 μL buffer, inject a 20 μL aliquot. (Prepare reagent by mixing 70 μL EtOH, 10 μL water, 10 μL triethylamine, and 10 μL phenylisothiocyanate just before use. Buffer was MeCN:water containing 710 mg Na_2HPO_4, adjust pH to 7.40 with phosphoric acid.)

HPLC VARIABLES

Column: 250 × 4.6 5 μm Ultrabase C18

Mobile phase: Gradient. A MeCN:buffer 6:76. A was MeCN:water 60:40. A:B from 100:0 to 54:46 over 14.5 min, to 0:100 over 0.5 min, return to initial conditions over 2 min, re-equilibrate at initial conditions for 3.5 min. (Buffer was 19 g/L sodium acetate trihydrate containing 500 μL/L triethylamine, adjust pH to 6.40 with glacial acetic acid.)

Column temperature: 38 ± 1

Flow rate: 1 for 15 min, to 1.5 over 2 min, maintain at 1.5 for 3 min, return to 1 over 0.5 min

Injection volume: 20

Detector: UV 254

CHROMATOGRAM

Retention time: 4.2 (Asp), 5 (Glu), 8.2 (Ser), 8.5 (Gly), 9 (His), 9.7 (Arg), 10.2 (Thr), 10.8 (Ala), 12.3 (Pro), 13.5 (Tyr), 14.4 (Val), 15.1 (Met), 15.6 (Cys), 16.5 (Ile), 16.7 (Leu), 17.5 (Phe), 17.7 (Trp), 17.9 (Lys)

KEY WORDS
derivatization

REFERENCE
Alonso,M.L.; Alvarez,A.I.; Zapico,J. Rapid analysis of free amino acids in infant foods, *J.Liq.Chromatogr.*, **1994**, *17*, 4019–4030.

SAMPLE
Matrix: formulations
Sample preparation: Dissolve formulations in 10-100 mM HCl, filter, analyze an aliquot. Peptides not containing Cys or Trp. Mix 500 μg peptide with 500 μL 6 M HCl, flush tube with nitrogen, heat at 110 ± 1° for 24 h, evaporate to dryness under a stream of nitrogen, reconstitute with 1 mL 100 mM HCl. Add a 2 μL aliquot to 5 μL 200 mM pH 10.4 borate buffer and 1 μL reagent, mix for 2 min, inject the whole amount. Peptides containing Trp. Hydrolyze 500 μg peptide with 500 μL 4 M methanesulfonic acid at 110° for 24 h, cool, add 500 μL water. Add a 2 μL aliquot to 5 μL 200 mM pH 10.4 borate buffer and 1 μL reagent, mix for 2 min, inject the whole amount. Peptides containing Cys. Dissolve 500 μg peptide in 1 mL formic acid, add 500 μL MeOH, cool to -10°, add 2.5 mL performic acid, let stand at -10° for 2.5 h, evaporate to dryness under reduced pressure, reconstitute with 500 μL 6 M HCl, flush tube with nitrogen, heat at 110 ± 1° for 24 h, evaporate to dryness under a stream of nitrogen, reconstitute with 1 mL 100 mM HCl. Add a 2 μL aliquot to 5 μL 200 mM pH 10.4 borate buffer and 1 μL reagent, mix for 2 min, inject the whole amount. (Prepare performic acid immediately before use by mixing 98% formic acid and 30% hydrogen peroxide in a 98:2 ratio, let stand for 2 h. Reagent was 260 mM N-isobutyryl-L-cysteine and 170 mM o-phthaldialdehydein 1 M potassium borate buffer (Pierce fluoraldehyde diluent).)

HPLC VARIABLES
Guard column: 20 × 2.1 5 μm Hypersil ODS
Column: 250 × 4 5 μm Hypersil ODS
Mobile phase: Gradient. A was 23 mM pH 6.0 sodium acetate buffer. B was MeOH:MeCN 60:5. A:B from 100:0 to 46.5:53.5 over 75 min.
Column temperature: 25
Flow rate: 1
Injection volume: 8
Detector: F ex 230 em 445

CHROMATOGRAM
Retention time: 19 (L-Asp), 20 (D-Asp), 26 (L-Glu), 28 (D-Glu), 35 (L-Thr), 37 (Gly), 38 (L-His), 42 (L-Ala), 44 (L-Arg), 45 (D-Arg), 45.5 (D-Ala), 56 (L-Val), 57 (L-Met), 59 (L-Trp), 61 (D-Val), 62 (L-Phe), 63 (L-Ile), 65 (D-Phe), 67 (L-Leu), 70 (D-Leu), 72 (L-Lys)

KEY WORDS
capsules; tablets; pills; dragees; granulates; derivatization; chiral

REFERENCE
Brückner,H.; Westhauser,T.; Godel,H. Liquid chromatographic determination of D- and L-amino acids by derivatization with *o*-phthaldialdehyde and N-isobutyryl-L-cysteine. Applications with reference to the analysis of peptidic antibiotics, toxins, drugs and pharmaceutically used amino acids, *J.Chromatogr.A*, **1995**, *711*, 201–215.

SAMPLE
Matrix: formulations
Sample preparation: Sonicate pills, capsules, powders, or drops in 50 mM sodium dodecyl sulfate, mix an aliquot with a 10-fold molar excess of reagent, let stand for 1 min, inject an aliquot. (Reagent was 2 mM o-phthalaldehyde and 2 mM N-acetyl-L-cysteine in 100 mM pH 9.5 sodium borate buffer.)

HPLC VARIABLES
Guard column: 35 × 4.6 5 μm Spherisorb ODS-2
Column: 120 × 4.6 5 μm Spherisorb ODS-2
Mobile phase: Propanol:50 mM sodium dodecyl sulfate 3:97, pH 3
Flow rate: 1
Injection volume: 20
Detector: UV 336

CHROMATOGRAM
Retention time: 2.5 (threonine), 3, 8 (lysine (different derivatives)), 3 (glycine), 6 (methionine)

KEY WORDS
pills; capsules; powders; drops; derivatization

REFERENCE
Catalá-Icardo,M.; Medina-Hernández,M.J.; García Alvarez-Coque,M.C. Determination of amino acids by micellar high-performance liquid chromatography and pre-column derivatization with o-phthalaldehyde and N-acetyl-L-cysteine, *J.Liq.Chromatogr.*, **1995**, *18*, 2827–2841.

SAMPLE
Matrix: fungal spore walls
Sample preparation: Hydrolyse fungal spore walls with 6 M HCl at 110° for 12 h, evaporate to dryness under reduced pressure, chromatograph on a DeltaPak C18 column (Waters) with 0.1% trifluoroacetic acid as mobile phase and detection at UV 214. Collect the unretained material and evaporate it to dryness under reduced pressure, dissolve 20 nmoles crude amino acids in 400 μL 100 mM pH 9 borate buffer, add 300 μL 10 mg/mL dansyl chloride in acetone, let stand in the dark for 2 h, evaporate to dryness under a stream of nitrogen, reconstitute with acetone:1 M HCl 95:5, centrifuge, evaporate the supernatant to dryness, repeat this extraction, reconstitute with mobile phase A, inject a 20 μL aliquot, elute to waste with mobile phase A, divert the fraction (200 μL) containing the amino acid from column A to column B (16.6-17 min for alanine; 24.5-24.8 min for glutamic acid), elute column B with mobile phase B, monitor the effluent from column B.

HPLC VARIABLES
Column: A 250 × 4 7 μm LiChrosorb RP-18; B 250 × 4 5 μm LiChrospher 100 RP-18
Mobile phase: A EtOH:25 mM pH 5.5 ammonium acetate containing 0.1% triethylamine 21:79 or 30:70; B EtOH:15 mM pH 5.5 ammonium acetate 20:80 containing 35 mM β-cyclodextrin and 1 M urea.
Flow rate: 0.5
Injection volume: 20
Detector: F ex 340 em 480

CHROMATOGRAM
Retention time: 12.5 (D-Glu (mobile phase A 21:79)), 13.3 (L-Glu (mobile phase A 21:79)), 38.6 (D-Ala (mobile phase A 30:70)), 41.5 (L-Ala (mobile phase A 30:70))

KEY WORDS
derivatization; chiral; column-switching

REFERENCE
Rizzi,A.M.; Briza,P.; Breitenbach,M. Determination of D-alanine and D-glutamic acid in biological samples by coupled-column chromatography using β-cyclodextrin as mobile phase additive, *J.Chromatogr.*, **1992**, *582*, 35–40.

SAMPLE
Matrix: hair, protein

Sample preparation: Suspend 10 mg protein or hair in 10 mL 6 M HCl, seal tube under vacuum, heat at 110° for 20 h. Remove a 1 mL aliquot and evaporate it to dryness, dissolve the residue in 1 mL 100 mM HCl, make up to 10 mL with water, filter (Adovantex DISMIC-13cp), dilute the filtrate 10-fold with water. Place a 100 μL aliquot of 100 μg/mL solution of 1-methoxycarbonylindolizine-3,5-dicarbaldehyde in ethyl acetate in the bottom of a tube, evaporate to dryness under reduced pressure, add 100 μL reaction buffer, sonicate for 30 s, add 20 μL protein hydrolysate, mix well, let stand for 20 min in the dark, inject a 10 μL aliquot. (Prepare phosphate-borate buffer by mixing equal volumes of 20 mM NaH_2PO_4 and 20 mM sodium tetraborate, adjust pH to 10 with 1 M NaOH. Prepare reaction buffer by mixing equal volumes of EtOH and phosphate-borate buffer. Prepare reagent (1-methoxycarbonylindolizine-3,5-dicarbaldehyde) as follows. Reflux 21.4 g 2-pyridinecarboxaldehyde, 24 mL ethylene glycol, 10 g p-toluenesulfonic acid, and 300 mL benzene (Caution! Benzene is a carcinogen!) under a Dean-Stark separator for 64 h, pour into concentrated sodium carbonate solution. Remove the organic layer and extract the aqueous layer 4 times with benzene. Combine the organic layers and wash them with water, dry over anhydrous magnesium sulfate, evaporate, distil the residue to give 2-(1,3-dioxolan-2-yl)pyridine (bp 122°/4 mm Hg) (J.Org.Chem. 1963, 28, 83). Reflux 15.1 g 2-(1,3-dioxolan-2-yl)pyridine and 19.5 g tert-butyl bromoacetate in 100 mL dry acetonitrile for 7 h,let stand overnight at room temperature, filter, wash the precipitate with diethyl ether to give 1-(tert-butoxycarbonylmethyl)-2-(1,3-dioxolan-2-yl)pyridinium bromide (mp 110-2° from MeCN). Suspend 51.9 g of this compound in 1.5 L THF with stirring, add 62.1 g potassium carbonate, add 15.12 g methyl propiolate, stir at room temperature for 9 days, filter, evaporate the filtrate to dryness under reduced pressure, chromatograph the residue on silica gel with hexane:ethyl acetate 20:1-10:1, collect fractions and evaporate to dryness to give methyl 3-tert-butoxycarbonyl-5-(1,3-dioxolan-2-yl)indolizine-1-carboxylate (mp 138-9° from hexane). Reflux 20.82 g of this compound in 600 mL THF and 60 mL 10% HCl for 6 h, concentrate to one quarter of the original volume, add water, extract with chloroform. Wash the chloroform layer with water and dry it over anhydrous sodium sulfate, evaporate, chromatograph on silica gel with hexane:ethyl acetate 10:1 to give 1-methoxycarbonylindolizine-5-carbaldehyde (mp 135-7° from MeOH). Stir 12.18 g of this compound in 116 mL dry DMF at 0° under argon, add 17 mL phosphorus oxychloride, stir at room temperature for 1 h, pour into water, adjust pH to 9.0 with 5% potassium carbonate, extract with chloroform. Wash the organic layer with water and dry it over anhydrous sodium sulfate, concentrate until a precipitate forms, filter to obtain the product, concentrate the filtrate and chromatograph the residue on silica gel with hexane:ethyl acetate 5:1 to obtain more product. The product was 1-methoxycarbonylindolizine-3,5-dicarbaldehyde (mp 164-5° from methyl acetate).)

HPLC VARIABLES

Column: 150 × 6 Asahipak ODP-50 (Asahi)

Mobile phase: Gradient. MeCN:buffer from 7:93 to 20:80 over 40 min, to 30:70 over 10 min, to 50:50 over 5 min, return to initial conditions over 5 min.) (Prepare buffer by adjusting the pH of 20 mM $(NH_4)H_2PO_4$ containing 10 mM sodium 1-octanesulfonate to 2.6 with phosphoric acid.)

Column temperature: 40

Flow rate: 1

Injection volume: 10

Detector: F ex 414 em 482

CHROMATOGRAM

Retention time: 13.8 (Asp), 15.5 (Ser), 17 (Gly), 18.8 (Glu), 19.2 (Cys), 20 (Thr), 21.5 (Ala), 30 (His), 33.8 (Val), 34.6 (ammonia), 35.8 (Met), 38.3 (Arg), 39.2 (Lys), 42.5 (Ile), 44.2 (Leu), 46.7 (Phe)

Limit of detection: 0.2-200 fmole

KEY WORDS

derivatization; soybean

REFERENCE

Oguri,S.; Uchida,C.; Mishina,M.; Miki,Y.; Kakehi,K. Determination of amino acids by pre-column fluorescence derivatization with 1-methoxycarbonylindolizine-3,5-dicarbaldehyde, *J.Chromatogr.A*, **1996**, *724*, 169–177.

SAMPLE

Matrix: peptides

Sample preparation: Freeze-dry 0.1-1 μg peptide containing 1-10 nmole total amino acids, add 20 μL 6 M HCl, seal in a tube under reduced pressure, heat at 110° for 24 h, evaporate under vacuum, add 10 μL 100 mM pH 9.0 sodium bicarbonate buffer, add 20 μL 4 mM dimethylaminoazobenzenesulfonyl chloride (dabsyl chloride) in acetone, heat at 70° for 10-15 min with occasional shaking, dilute to 100-500 μL with EtOH:water 70:30, inject a 10 μL aliquot. (Recrystallize dimethylaminoazobenzenesulfonyl chloride from acetone.)

HPLC VARIABLES

Column: Zorbax ODS

Mobile phase: Gradient. G1 = MeCN:buffer from 20:80 to 70:30 over 25 min, stay at 70:30 for 5 min, wash with 100:0 for 15 min, return to initial conditions over 5 min, re-equilibrate for 10 min. (Buffer was 5.44 g sodium acetate trihydrate and 7.7 mL acetic acid made up to 900 mL with water, pH 4.13.) (Using G2 = MeCN:pH 7.2 phosphate buffer from 20:80 to 35:65 over 15 min, stay at 35:65 for 5 min, go to 65:35 over 5 min, stay at 65:35 for 5 min, Asp and Ser are separated but other separations are not as good.)

Flow rate: 1.2

Injection volume: 10

Detector: UV 436

CHROMATOGRAM

Retention time: 13.5 (8.5) (Asp) (for G1, G2 times in parentheses), 13.5 (14.5) (Ser), 14 (9) (Glu), 14.5 (15) (Thr), 15 (15.5) (Gly), 16 (18) (Arg), 17 (16) (Ala), 19 (18.5) (Met), 20 (17) (Pro), 20.5 (17.5) (Val), 21 (21) (Phe), 22.5 (19.2) (Leu), 23 (18.8) (Ile), 26 (27.3) (His), 27 (27) (Lys), 30 (28) (Tyr)

KEY WORDS

derivatization; dabsylation; dabsyl; Asp and Ser co-elute

REFERENCE

Chang,J.-Y.; Knecht,R.; Braun,D.G. Amino acid analysis at the picomole level. Application to the C-terminal sequence analysis of polypeptides, *Biochem.J.*, **1981**, *199*, 547–555.

SAMPLE

Matrix: peptides

Sample preparation: Freeze dry 0.1-1 μg peptide in a tube, add 20 μL 6 M HCl, seal under reduced pressure, heat at 120° for 24 h, centrifuge, evaporate to dryness, reconstitute with 10 μL 200 mM pH 9.0 sodium bicarbonate buffer, add 20 μL 4 mM dabsyl chloride in acetone, heat at 70° with occasional shaking for 10-15 min, dilute to 100-500 μL with EtOH:water 70:30 or MeCN:water 50:50, inject a 10 μL aliquot.

HPLC VARIABLES

Column: Lichrosorb C-18

Mobile phase: Gradient. A was DMF:17 mM pH 6.5 phosphate buffer 2:98. B was MeCN:DMF 96:4. A:B from 85:15 to 55:45 over 25 min, to 30:70 over 10 min.

Column temperature: 50

Flow rate: 1

Injection volume: 10

Detector: UV 436